FRESHWATER BIOLOGY AND POLLUTION ECOLOGY

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER PROGRAMS

FRESHWATER BIOLOGY

This course is designed as an introduction to aquatic biology for sanitary engineers, scientists, and others who are involved in freshwater pollution studies, surveillance, and control. Biologists new to the field of aquatic biology and pollution problems may find it useful for orientation.

AND

FRESHWATER POLLUTION ECOLOGY

This course is offered for aquatic biologists or persons with comparable experience concerned with and/or involved in the application of biological principles, techniques and parameters to pollution studies and abatement programs

ENVIRONMENTAL PROTECTION AGENCY
Water Programs Operations
TRAINING PROGRAM

April 1973

TRAINING PROGRAM

Through the Water Programs Operations Office, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and the responsibility of the states in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the states is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be available through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the states for their own further production and presentation.

A bulletin of courses is prepared and distributed periodically by the National Training Center. The bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from The National Training Center.

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Programs, Environmental Protection Agency.

Due to the limited availability of the manuals, it is not appropriate to cite them as technical references in bibliographies or other forms of publication.

References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Programs, Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

Joseph Bahnick
Acting Chief
Direct Technical Training Branch
Division of Manpower and Training
Office of Water Programs
Environmental Protection Agency

COURSE DESCRIPTION

FRESHWATER BIOLOGY AND POLLUTION ECOLOGY (140)

2 weeks

CINCINNATI, OHIO June 4-15, 1973

This course is designed for technical personnel (other than biologists) engaged in water quality analysis and management

Within the framework of his personal background capacities, and experience, the student should be able, on completion of this course to

Understand many basic environmental factors impinging on aquatic communities

Recognize or identify to broad groups most freshwater organisms commonly encountered, using correct procedures and appropriate literature when available and also using judgment in assesing his own technical capacity in regard to the degree of identification attempted

Select and use appropriate common types of biological field collection equipment and procedures

Select and use appropriate types of biological laboratory analytical equipment and procedures

Perform simple analyses of an aquatic community in order to assess the likelihood that it may have been disturbed by pollution

Recognize gross biological indications of particular types of pollution when present

Predict possible effects of a given type of pollutant on a given habitat

Organize a field survey to determine the severity and extent of pollution.

Course work includes lectures, discussions, problem assignments, and laboratory sessions. Field work is included to allow student participation in selecting and using biological field collection equipment and familiarization with biological communities.

Representative topics usually include:

Types of aquatic organisms

Aquatic organisms or significance in water quality

Lake, reservoir, and stream sampling techniques

Use of artificial substrates

Thermal pollution

Investigation of fish kills

Environmental quality

Eutrophication in the freshwater environment

Biological magnification

Participants should bring appropriate clothing for field work, including rainwear. Boots will be supplied locally unless notice is given to the contrary.

U.S. Environmental Protection Agency

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CONTENTS

SURVEY OF THE BIOTA

The first half of this manual is a synoptic review of the components of the aquatic community to aid the observer in recognizing many common types of organisms encountered both in the field, laboratory, and treatment facilities.

A comprehension of the system of biological nomenclature and ecological classifications, is basic to an understanding of aquatic life communities. The first exercise in ecology is systematics. Application of systematics will depend on one's background, present limitations, and program objectives.

Systematics, broadly defined, is the study of the diversity of organisms. In connotation it is a wedding between taxonomy and ecology. In applied biology or ecology, good systematics is indispensable and is an immensely useful system of information storage and retrieval. The following definitions are basic.

SYSTEMATICS: "The scientific study of the kinds and diversity of organisms and of any and all relationships among them."

CLASSIFICATION: "The ordering of organisms into groups (or sets) on the basis of their relationships, that is, of their associations by contiguity, similarity, or both."

TAXONOMY: "The theoretical study of classification, including its bases, principles, procedures, and rules."

IDENTIFICATION: "The use of a key (or key substitute like an expert) to place an unknown organism into a specific taxonomic rank."

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THE AQUATIC ENVIRONMENT

Part 1: The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters.

A Upon the hydrosphere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it.

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4°C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H^+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3, ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

2 Density

a Temperature and density Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

SUBSTANCE OF WATER

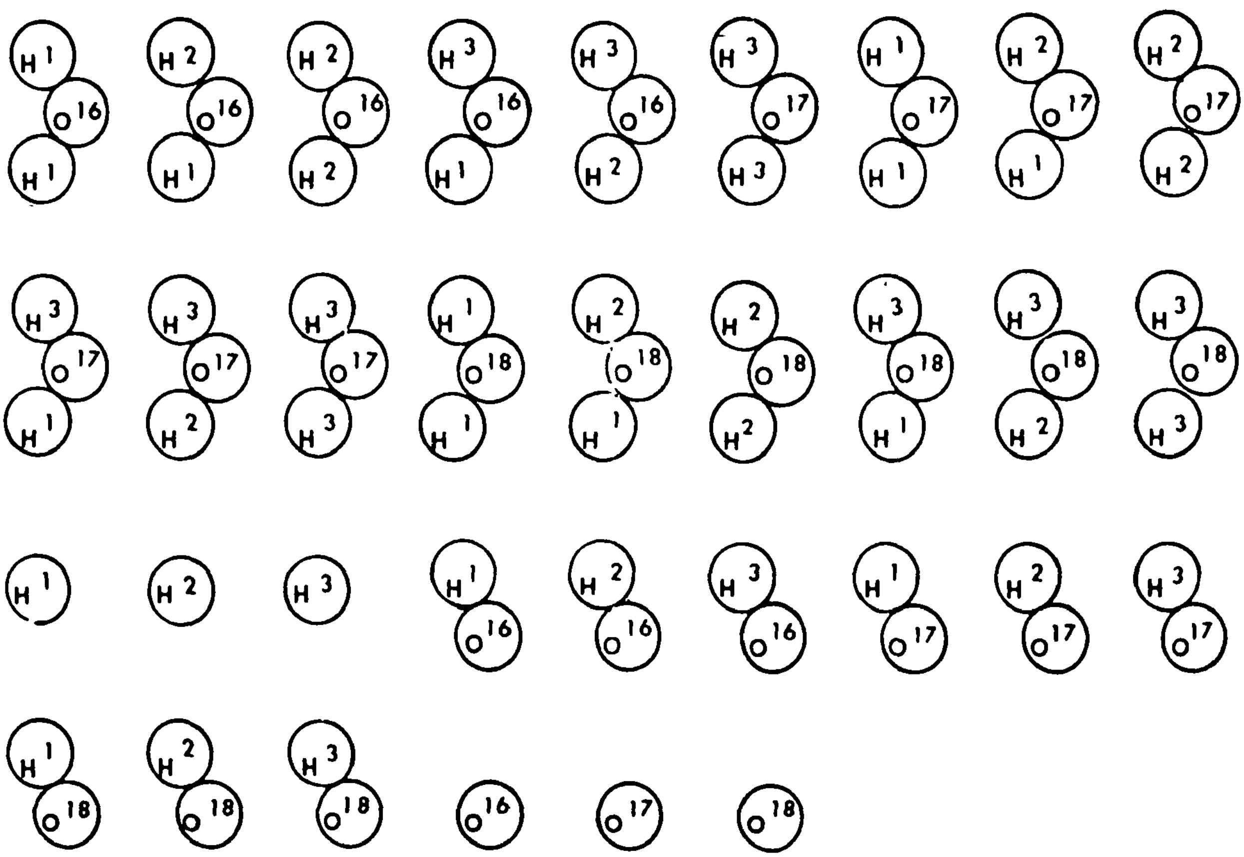

Figure 1

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (° C)	Density Water	Ice **
-10	.99815	.9397
- 8	99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.00997	
8	.00988	
10	.00973	

* Tabular values for density, etc., represent statistical estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice.

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers: the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a thermocline is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures (and probably other qualities too) may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) It should also be emphasized that when epilimnion and hypolimnion achieve the same temperature, stratification no longer exists, and the entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Such periods are called overturns and

usually result in considerable water quality changes of physical, chemical, and biological significance.

6) When stratification is present, however, each layer behaves relatively independently, and considerable quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass, it is found in oceans and puddles

8) The relative densities of the various isotopes of water also influence its molecular composition For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{16} content in warmer climates Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

b Dissolved and/or suspended solids may also affect the density of natural waters

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

c Density caused stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above. The spring and fall overturns of such waters materially affect biological productivity.

5) Density stratification is not limited to two-layered systems, three, four, or even more layers may be encountered in larger bodies of water.

d A "plunge line" may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

2 The viscosity of water is greater at lower temperatures (see Table 4). This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm tropical waters.

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

Temp. ° C	Dissolved solids in g/L			
	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

3 Surface tension has biological as well as physical significance Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water

4 Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

5 Water movements

a Waves or rhythmic movement

The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water such that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

Tides are the longest waves known in the ocean, and are evident along the coast by the rhythmic rise and fall of the water. While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide", and to the accompanying currents as "tidal currents"

Tides are basically caused by the attraction of the sun and moon on water masses, large and small, however, it is only in the oceans and certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady a rhythmic water movements which have had major study only in oceanography although they are best known from rivers and streams. They primarily are concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuir circulation (or L. spirals) is the interlocking rotation of somewhat cylindrical masses of surface water under the influence of wind action. The axes of the cylinders are parallel to the direction of the wind.

To somewhat oversimplify the concept, a series of adjoining cells might be thought of as chains of interlocking gears in which at every other contact the teeth are rising while at the alternate contacts, they are sinking (Figure 2).

The result is elongated masses of waste rising or sinking together. This produces the familiar "wind rows" of foam, flotsam and jetsam, or plankton often seen streaking windblown lakes or oceans. Certain zoo-plankton struggling to maintain a position near the surface often collect in the down current between two Langmuir cells, causing such an area to be called the "red dance", while the clear upwelling water between is the "blue dance".

This phenomenon may be important in water or plankton sampling on a windy day.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality Other items not specifically mentioned include· molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, ices, phenomena of hydrostatics and hydrodynamics in general.

REFERENCES

1 Buswell, A.M. and Rodebush, W.H. Water. Sci. Am. April 1956.

2 Dorsey, N. Ernest. Properties of Ordinary Water - Substance. Reinhold Publ. Corp. New York. pp. 1-673. 1940.

3 Hutcheson, George E. A Treatise on Limnology. John Wiley Company. 1957.

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

THE AQUATIC ENVIRONMENT

Part 2 The Aquatic Environment as an Ecosystem

I INTRODUCTION

Part 1 introduced the lithosphere and the hydrosphere. Part 2 will deal with certain general aspects of the biosphere, or the sphere of life on this earth, which photographs from space have shown is a finite globe in infinite space.

This is the habitat of man and the other organisms. His relationships with the aquatic biosphere are our common concern.

II THE BIOLOGICAL NATURE OF THE WORLD WE LIVE IN

A We can only imagine what this world must have been like before there was life.

B The world as we know it is largely shaped by the forces of life.

1 Primitive forms of life created organic matter and established soil.

2 Plants cover the lands and enormously influence the forces of erosion.

3 The nature and rate of erosion affect the redistribution of materials (and mass) on the surface of the earth (topographic changes).

4 Organisms tie up vast quantities of certain chemicals, such as carbon and oxygen.

5 Respiration of plants and animals releases carbon dioxide to the atmosphere in influential quantities.

6 CO_2 affects the heat transmission of the atmosphere.

C Organisms respond to and in turn affect their environment. Man is one of the most influential.

III ECOLOGY IS THE STUDY OF THE INTERRELATIONSHIPS BETWEEN ORGANISMS, AND BETWEEN ORGANISMS AND THEIR ENVIRONMENT.

A The ecosystem is the basic functional unit of ecology. Any area of nature that includes living organisms and nonliving substances interacting to produce an exchange of materials between the living and nonliving parts constitutes and ecosystem. (Odum, 1959)

1 From a structural standpoint, it is convenient to recognize four constituents as composing an ecosystem (Figure 1).

a Abiotic NUTRIENT NUMERALS which are the physical stuff of which living protoplasm will be synthesized.

b Autotrophic (self-nourishing) or PRODUCER organisms. These are largely the green plants (holophytes), but other minor groups must also be included (See Figure 2). They assimilate the nutrient minerals, by the use of considerable energy, combine them into living organic substance.

c Heterotrophic (other-nourishing) CONSUMERS (holozoic), are chiefly the animals. They ingest (or eat) and digest organic matter, releasing considerable energy in the process.

d Heterotrophic REDUCERS are chiefly bacterial and fungi that return complex organic compounds back to the original abiotic mineral condition, thereby releasing the remaining chemical energy.

2 From a functional standpoint, an ecosystem has two parts (Figure 2)

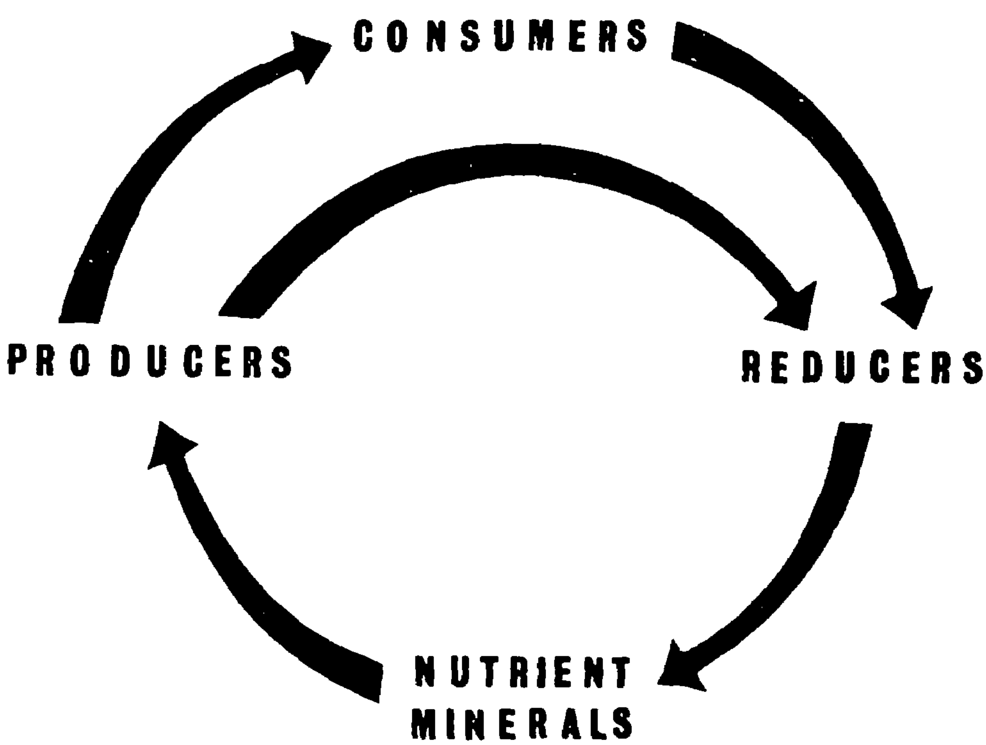

FIGURE 1

a The autotrophic or producer organisms, which construct organic substance.

b The heterotrophic or consumer and reducer organisms which destroy organic substance.

3 Unless the autotrophic and heterotrophic phases of the cycle approximate a dynamic equilibrium, the ecosystem and the environment will change.

B Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms. (Figure 2)

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms. They are collectively called the PROTISTA.

2 Within the protista, two principal subgroups can be defined on the basis of relative complexity of structure.

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera).

b The single-celled algae and protozoa are best referred to as the higher protista.

C Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

IV FUNCTIONING OF THE ECOSYSTEM

A A food chain is the transfer of food energy from plants through a series of organisms with repeated eating and being eaten. Food chains are not isolated sequences but are interconnected.

RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS

Energy Flows from Left to Right, General Evolutionary Sequence is Upward

PRODUCERS	CONSUMERS		REDUCERS
Organic Material Produced, Usually by Photosynthesis	Organic Material Ingested or Consumed Digested Internally		Organic Material Reduced by Extracellular Digestion and Intracellular Metabolism to Mineral Condition
ENERGY STORED	ENERGY RELEASED		ENERGY RELEASED
Flowering Plants and Gymnosperms	Arachnids	Mammals	Basidiomycetes
	Insects	Birds	
Club Mosses, Ferns	Crustaceans	Reptiles	
	Segmented Worms	Amphibians	
Liverworts, Mosses	Molluscs	Fishes	Fungi Imperfecti
Multicellular Green Algae	Bryozoa	Primitive Chordates	
	Rotifers		Ascomycetes
	Roundworms	Echinoderms	
Red Algae	Flatworms		
	Coelenterates		Higher Phycomycetes
Brown Algae	Sponges		

DEVELOPMENT OF MULTICELLULAR OR COENOCYTIC STRUCTURE

H I G H E R P R O T I S T A

	Protozoa		
Unicellular Green Algae	Amoeboid	Ciliated	Lower Phycomycetes
Diatoms	Flagellated, (non-pigmented)	Suctoria	(Chytridiales, et al)
Pigmented Flagellates			

DEVELOPMENT OF A NUCLEAR MEMBRANE

L O W E R P R O T I S T A

(or Monera)

Blue Green Algae	Actinomycetes
Phototropic Bacteria	Spirochaetes
Chemotropic Bacteria	Saprophytic Bacterial Types

FIGURE 2

BI ECO pl 2a 1 69

B A food web is the interlocking pattern of food chains in an ecosystem. (Figures 3, 4) In complex natural communities, organisms whose food is obtained by the same number of steps are said to belong to the same trophic (feeding) level.

C Trophic Levels

1 First - Green plants (producers) (Figure 5) fix biochemical energy and synthesize basic organic substances.

2 Second - Plant eating animals (herbivores) depend on the producer organisms for food.

3 Third - Primary carnivores, animals which feed on herbivores.

4 Fourth - Secondary carnivores feed on primary carnivores.

5 Last - Ultimate carnivores are the last or ultimate level of consumers.

D Total Assimilation

The amount of energy which flows through a trophic level is distributed between the production of biomass and the demands of respiration in a ratio of approximately 1:10.

E Trophic Structure of the Ecosystem

The interaction of the food chain phenomena (with energy loss at each transfer) results in various communities having definite trophic structure or energy levels. Trophic structure may be measured and described either in terms of the standing crop per unit area or in terms of energy fixed per unit area per unit time at successive trophic levels. Trophic structure and function can be shown graphically by means of ecological pyramids (Figure 5).

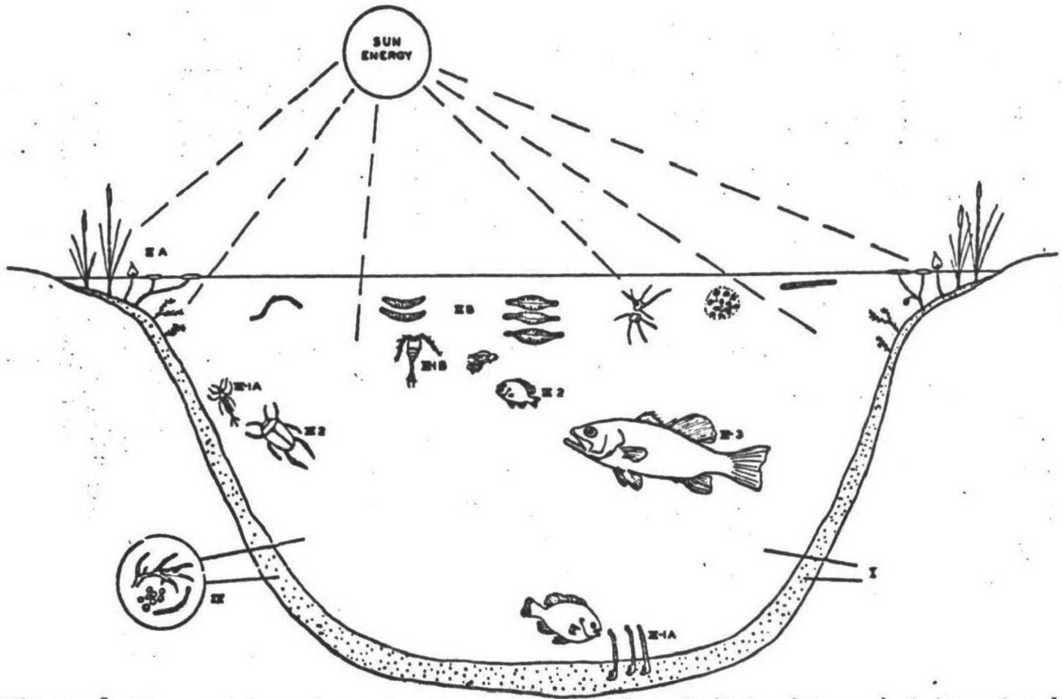

Figure 3. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances–basic inorganic and organic compounds; IIA, producers–rooted vegetation; IIB, producers–phytoplankton; III-1A, primary consumers (herbivores)–bottom forms; III-1B, primary consumers (herbivores)–zooplankton; III-2, secondary consumers (carnivores); III-3, tertiary consumers (secondary carnivores); IV, decomposers–bacteria and fungi of decay.

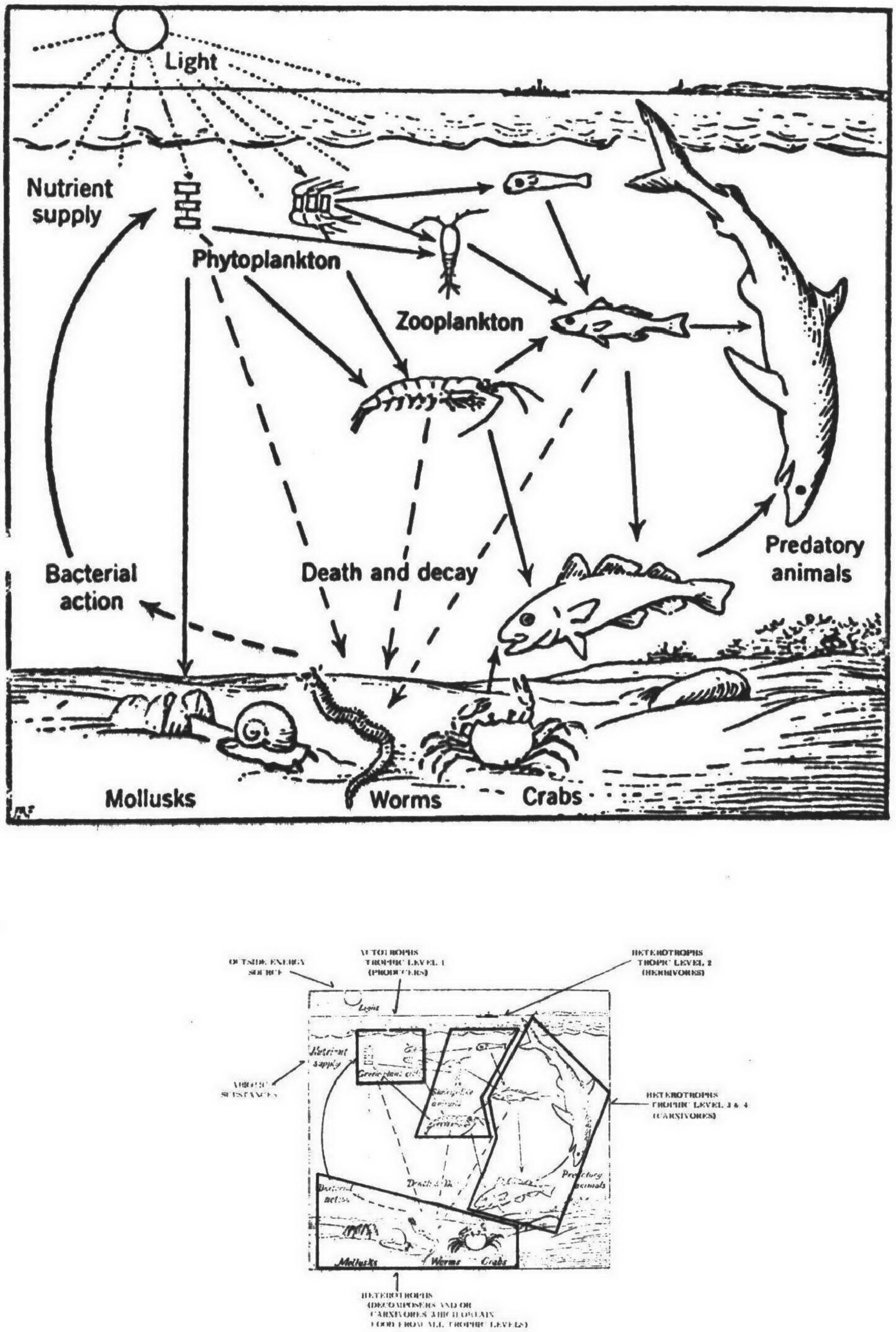

Figure 4. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

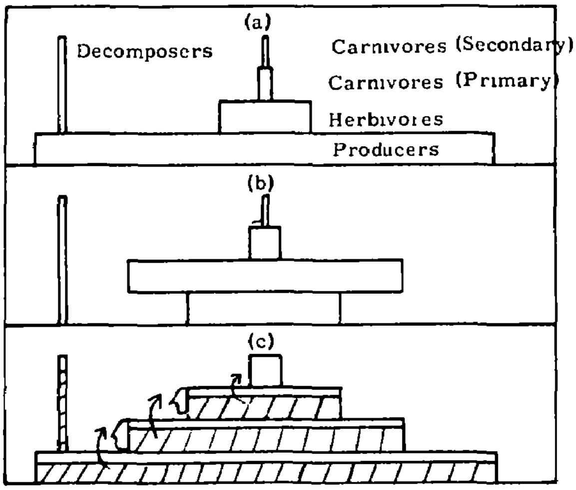

Figure 5. HYPOTHETICAL PYRAMIDS of (a) Numbers of Individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

V BIOTIC COMMUNITIES

A Plankton are the macroscopic and microscopic animals, plants, bacteria, etc., floating free in the open water. Many clog filters, cause tastes, odors, and other troubles in water supplies. Eggs and larvae of larger forms are often present.

1 Phytoplankton are plant-like. These are the dominant producers of the waters, fresh and salt, "the grass of the seas".

2 Zooplankton are animal-like. Includes many different animal types, range in size from minute protozoa to gigantic marine jellyfishes.

B Periphyton (or Aufwuchs) - The communities of microscopic organisms associated with submerged surfaces of any type or depth. Includes bacteria, algae, protozoa, and other microscopic animals, and often the young or embryonic stages of algae and other organisms that normally grow up to become a part of the benthos (see below). Many planktonic types will also adhere to surfaces as periphyton, and some typical periphyton may break off and be collected as plankters.

C Benthos are the plants and animals living on, in, or closely associated with the bottom. They include plants and invertebrates.

D Nekton are the community of strong aggressive swimmers of the open waters, often called pellagic. Certain fishes, whales, and invertebrates such as shrimps and squids are included here.

E The marsh community is based on larger "higher" plants, floating and emergent. Both marine and freshwater marshes are areas of enormous biological production.

VI PRODUCTIVITY

A The biological resultant of all physical and chemical factors is the quantity of life that may actually be present. The ability to produce this "biomass" is often referred to as the "productivity" of a body of water. This is neither good nor bad per se. A water of low productivity is a "poor" water biologically, and also a relatively "pure" or "clean" water, hence desirable as a water supply or a bathing beach. A productive water on the other hand may be a nuisance to man or highly desirable. It is a nuisance if foul odors and/or weed-chocked waterways result, it is desirable if bumper crops of bass, catfish, or oysters are produced. Open oceans on the other hand have a very low level of productivity in general

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THE AQUATIC ENVIRONMENT

Part 3. The Freshwater Environment

I INTRODUCTION

The freshwater environment as considered herein refers to those inland waters not detectably diluted by ocean waters, although the lower portions of rivers are subject to certain tidal flow effects.

Certain atypical inland waters such as saline or alkaline lakes, springs, etc., are not treated, as the main objective is typical inland water.

All waters have certain basic biological cycles and types of interactions most of which have already been presented. Hence this outline will concentrate on aspects essentially peculiar to fresh inland waters.

II PRESENT WATER QUALITY AS A FUNCTION OF THE EVOLUTION OF FRESH WATERS

A The history of a body of water determines its present condition. Natural waters have evolved in the course of geologic time into what we know today.

B Streams

In the course of their evolution, streams in general pass through four general stages of development which may be called. birth, youth, maturity, and old age.

1 Establishment or birth. In an extant stream, this might be a "dry run" or headwater stream-bed, before it had eroded down to the level of ground water.

2 Youthful streams, when the stream-bed is eroded below the ground water level, spring water enters and the stream becomes permanent.

3 Mature streams, have wide valleys, a developed flood plain, deeper, more turbid, and usually warmer water, sand, mud, silt, or clay bottom materials which shift with increase in flow.

4 In old age, streams have approached geologic base level. During flood stage they scour their beds and deposit materials on the flood plain which may be very broad and flat. During normal flow the channel is refilled and many shifting bars are developed. (Under the influence of man this pattern may be broken up, or temporarily interrupted. Thus an essentially "youthful" stream might take on some of the characteristics of a "mature" stream following soil erosion, organic enrichment, and increased surface runoff. Correction of these conditions might likewise be followed by at least a partial reversion to the "original" condition).

C Lakes and Reservoirs

Geological factors which significantly affect the nature of either a stream or lake include the following

1 The geographical location of the drainage basin or watershed.

2 The size and shape of the drainage basin.

3 The general topography, i.e., mountainous or plains.

4 The character of the bedrocks and soils.

5 The character, amount, annual distribution, and rate of precipitation.

6 The natural vegetative cover of the land is of course responsible to many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining runoff versus soil absorption, etc.

D Lakes have a developmental history which somewhat parallels that of streams.

1 The method of formation greatly influences the character and subsequent history of lakes.

2 Maturing or natural eutrophication of lakes.

a If not already present shoal areas are developed through erosion of the shore by wave action and undertow.

b Currents produce bars across bays and thus cut off irregular areas.

c Silt brought in by tributary streams settles out in the quiet lake water.

d Rooted aquatic plants grow on shoals and bars, and in doing so cut off bays and contribute to the filling of the lake.

e Dissolved carbonates and other materials are precipitated in the deeper portions of the lake in part through the action of plants.

f When filling is well advanced, mats of sphagnum moss may extend outward from the shore. These mats are followed by sedges and grasses which finally convert the lake into a marsh.

3 Extinction of lakes. After lakes reach maturity, their progress toward filling up is accelerated. They become extinct through

a The downcutting of the outlet.

b Filling with detritus eroded from the shores or brought in by tributary streams.

c Filling by the accumulation of the remains of vegetable materials growing in the lake itself. (Often two or three processes may act concurrently)

III PRODUCTIVITY IN FRESH WATERS

A Fresh waters in general and under natural conditions by definition have a lesser supply of dissolved substances than marine waters, and thus a lesser basic potential for the growth of aquatic organisms. By the same token, they may be said to be more sensitive to the addition of extraneous materials (pollutants, nutrients, etc.) The following notes are directed toward natural geological and other environmental factors as they affect the productivity of fresh waters.

B Factors Affecting Stream Productivity (See Table 1)

TABLE 1

EFFECT OF SUBSTRATE ON STREAM PRODUCTIVITY*

(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Marl	6
Fine Gravel	9
Gravel and silt	14
Coarse gravel	32
Moss on fine gravel	89
Fissidens (moss) on coarse gravel	111
Ranunculus (water buttercup)	194
Watercress	301
Anacharis (waterweed)	452

*Selected from Tarzwell 1937

To be productive of aquatic life, a stream must provide adequate nutrients, light, a suitable temperature, and time for growth to take place.

1 Youthful streams, especially on rock or sand substrates are low in essential nutrients. Temperatures in mountainous regions are usually low, and due to the steep gradient, time for growth is short. Although ample light is available, growth of true plankton is thus greatly limited.

2 As the stream flows toward a more "mature" condition, nutrients tend to accumulate, and gradient diminishes and so time of flow increases, temperature tends to increase, and plankton flourish.

Should a heavy load of inert silt develop on the other hand, the turbidity would reduce the light penetration and consequently the general plankton production would diminish.

3 As the stream approaches base level (old age) and the time available for plankton growth increases, the balance between turbidity, nutrient levels, and temperature and other seasonal conditions, determines the overall productivity.

C Factors Affecting the Productivity of Lakes

1 The size, shape, and depth of the lake basin. Shallow water is more productive than deeper water since more light will reach the bottom to stimulate rooted plant growth. As a corollary, lakes with more shoreline, having more shallow water, are in general more productive. Broad shallow lakes and reservoirs have the greatest production potential (and hence should be avoided for water supplies).

2 Hard waters are generally more productive than soft waters as there are more plant nutrient minerals available. This is often greatly influenced by the character of the soil and rocks in the watershed and the quality and quantity of ground water entering the lake. In general, pH ranges of 6.8 to 8.2 appear to be most productive.

TABLE 2

EFFECT OF SUBSTRATE
ON LAKE PRODUCTIVITY *
(The productivity of sand bottoms is taken as 1)

Bottom Material	Relative Productivity
Sand	1
Pebbles	4
Clay	8
Flat rubble	9
Block rubble	11
Shelving rock	77

*Selected from Tarzwell 1937

3 Turbidity reduces productivity as light penetration is reduced.

4 The presence or absence of thermal stratification with its semi-annual turnovers affects productivity by distributing nutrients throughout the water mass.

5 Climate, temperature, prevalence of ice and snow, are also of course important.

D Factors Affecting the Productivity of Reservoirs

1 The productivity of reservoirs is governed by much the same principles as that of lakes, with the difference that the water level is much more under the control of man Fluctuations in water level can be used to deliberately increase or decrease productivity. This can be demonstrated by a comparison of the TVA reservoirs which practice a summer drawdown with some of those in the west where a winter drawdown is the rule.

2 The level at which water is removed from a reservoir is important to the productivity of the stream below

The hypolimnion may be anaerobic while the epilimnion is aerobic, for example, or the epilimnion is poor in nutrients while the hypolimnion is relatively rich.

3 Reservoir discharges also profoundly affect the DO, temperature, and turbidity in the stream below a dam. Too much fluctuation in flow may permit sections of the stream to dry, or provide inadequate dilution for toxic waste.

VII CLASSIFICATION OF LAKES AND RESERVOIRS

A The productivity of lakes and impoundments is such a conspicuous feature that it is often used as a convenient means of classification.

1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

2 Eutrophic lakes are more mature, more turbid, and richer They are usually shallower They are richer in dissolved solids, N, P, and Ca are abundant. Plankton is abundant and there is often a rich bottom fauna.

3 Dystrophic lakes, such as bog lakes, are low in pH, water yellow to brown, dissolved solids, N, P, and Ca scanty but humic materials abundant, bottom fauna and plankton poor, and fish species are limited.

B Reservoirs may also be classified as storage and run of the river.

1 Storage reservoirs have a large volume in relation to their inflow.

2 Run of the river reservoirs have a large flow-through in relation to their storage value.

C According to location, lakes and reservoirs may be classified as polar, temperate, or tropical. Differences in climatic and geographic conditions result in differences in their biology.

VIII SUMMARY

A A body of water such as a lake, stream, or estuary represents an intricately balanced system in a state of dynamic equilibrium. Modification imposed at one point in the system automatically results in compensatory adjustments at associated points.

B The more thorough our knowledge of the entire system, the better we can judge where to impose control measures to achieve a desired result.

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THE AQUATIC ENVIRONMENT

Part 4. The Marine Environment and its Role in the Total Aquatic Environment

I INTRODUCTION

A The marine environment is arbitrarily defined as the water mass extending beyond the continental land masses, including the plants and animals harbored within. This water mass is large and deep, covering about 70 percent of the earth's surface and being as deep as 7 miles. The salt content averages about 35 parts per thousand. Life extends to all depths.

B The general nature of the water cycle on earth is well known. Because the relatively large surface area of the earth is covered with water, roughly 70 percent of the earth's rainfall is on the seas. (Figure 1)

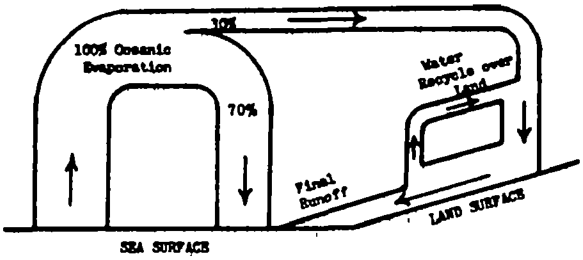


Figure 1. THE WATER CYCLE

Since roughly one third of the earth's rain which falls on the land is again recycled through the stratosphere (see Figure 1 again), the total amount of water washing over the earth's surface is significantly greater than one third of the total world rainfall. It is thus not surprising to note that the rivers which finally empty into the sea carry a considerable burden of dissolved and suspended solids picked up from the land. This is the substance of geological erosion.(Table 1)

TABLE 1

PERCENTAGE COMPOSITION OF THE MAJOR IONS OF TWO STREAMS AND SEA WATER

(Data from Clark, F.W., 1924, "The Composition of River and Lake Waters of the United States", U.S. Geol. Surv., Prof. Paper No. 135, Harvey, H.W., 1957, "The Chemistry and Fertility of Sea Waters", Cambridge University Press, Cambridge)

Ion	Delaware River at Lambertville, N.J.	Rio Grande at Laredo, Texas	Sea Water
Na	6 70	14.78	30 4
K	1.46	85	1.1
Ca	17.49	13.73	1.16
Mg	4 81	3.03	3 7
Cl	4 23	21 65	55 2
SO_4	17 49	30.10	7 7
CO_3	32.95	11.55	+HCO_3 0 35

C For this presentation, the marine environment will be (1) described using an ecological approach, (2) characterized ecologically by comparing it with freshwater and estuarine environments, and (3) considered as a functional ecological system (ecosystem).

II FRESHWATER, ESTUARINE, AND MARINE ENVIRONMENTS

Distinct differences are found in physical, chemical, and biotic factors in going from a freshwater to an oceanic environment. In general, environmental factors are more constant in freshwater (river) and oceanic environments when compared to the highly variable and harsh environments of estuarine and coastal waters.

A Physical and Chemical Factors (Figure 2)

1 Rivers

2 Estuary and coastal waters

3 Oceans

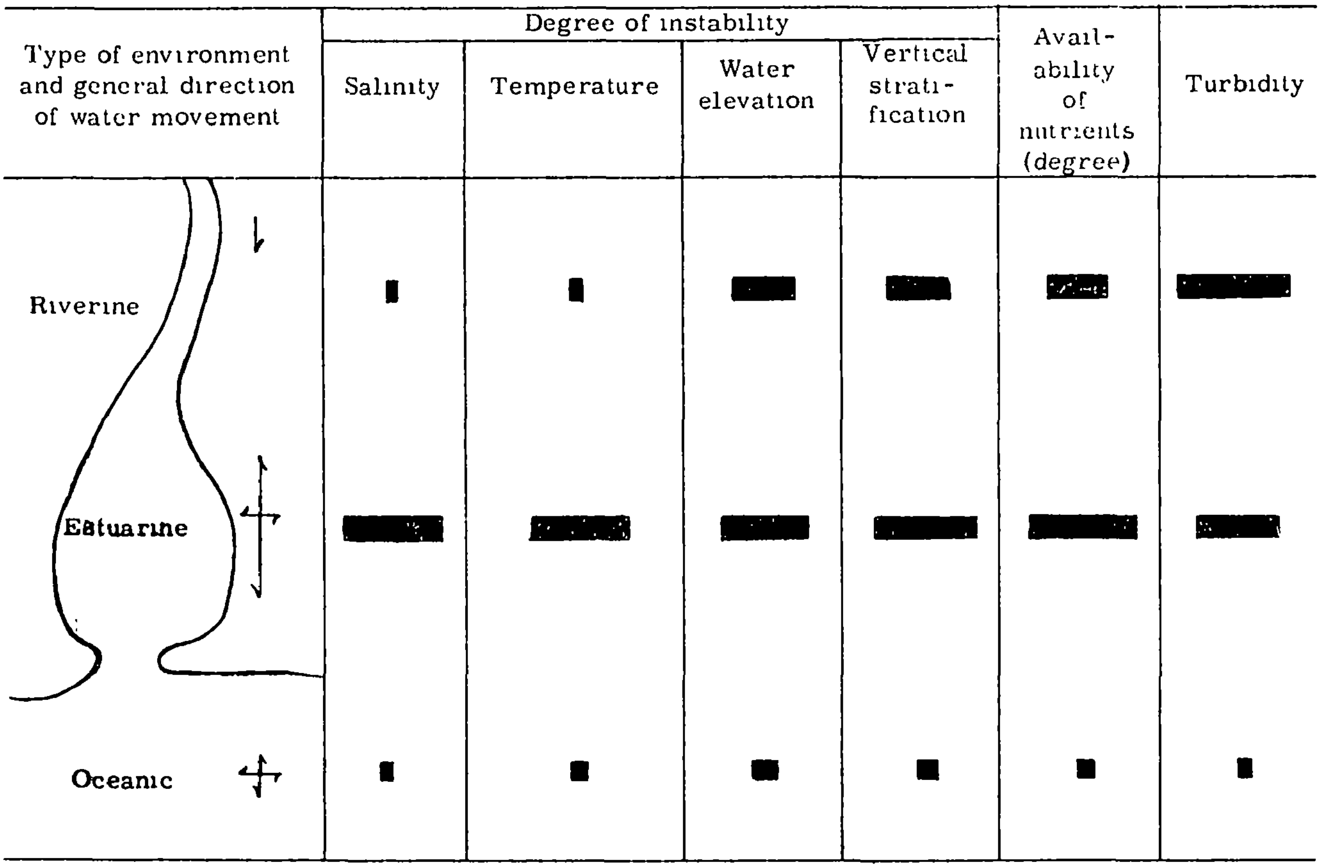


Figure 2. RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

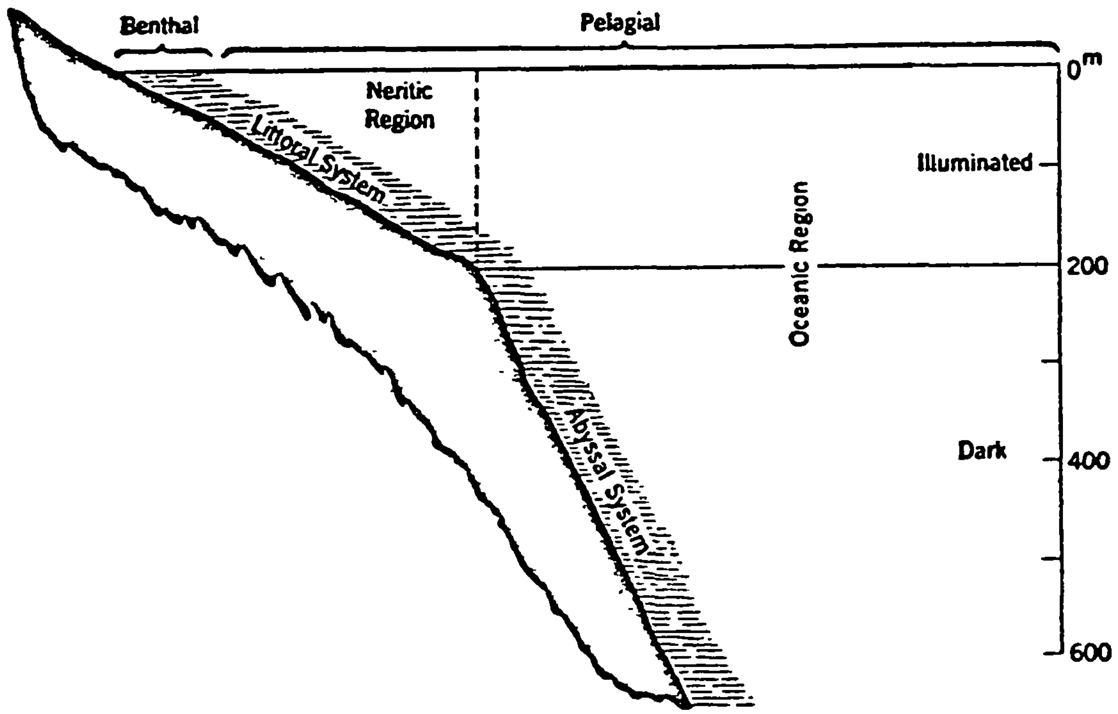
1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

2 The dominant animal species (in terms of total biomass) which occur in estuaries are often transient, spending only a part of their lives in the estuaries. This results in better utilization of a rich environment.

C Zones of the Sea

The nearshore environment is often classified in relation to tide level and water depth. The nearshore and oceanic regions together are often classified in relation to light penetration and water depth.

1 Neritic - Relatively shallow-water zone which extends from the high-tide mark to the edge of the continental shelf. (Figure 3)



Primary subdivisions of the marine habitat.

Figure 3.

a Stability of physical factors is intermediate between estuarine and oceanic environments.

b Phytoplankters are the dominant producers but in some locations attached algae are also important as producers.

c The animal consumers are zooplankton, nekton, and benthic forms.

2 Oceanic - The region of the ocean beyond the continental shelf. Divided into three parts, all relatively poorly populated compared to the neritic zone.

a Euphotic zone - Waters into which sunlight penetrates (often to the bottom in the neritic zone). The zone of basic productivity. Often extends to 600 feet below the surface.

1) Physical factors fluctuate less than in the neritic zone.

2) Producers are the phytoplankton and consumers are the zooplankton and nekton.

b Bathyal zone - From the bottom of the euphotic zone to about 6,000 feet.

1) Physical factors relatively constant but light is absent.

2) Producers are absent and consumers are scarce.

c Abyssal zone - All the sea below the bathyal zone.

1) Physical factors more constant than in bathyal zone.

2) Producers absent and consumers not as abundant as in the bathyal zone.

III SEA WATER AND THE BODY FLUIDS

A Sea water is a most suitable environment for living cells, because it contains all of the chemical elements essential to the growth and maintenance of plants and animals. The ratio and often the concentration of the major salts of sea water are strikingly similar in the cytoplasma and body fluids of marine organisms. This similarity is also evident, although modified somewhat in the body fluids of both fresh water and terrestrial animals. For example, sea water may be used in emergencies as a substitute for blood plasma in man.

B Since marine organisms have an internal salt content similar to that of their surrounding medium (isotonic condition) osmoregulation poses no problem. On the other hand, fresh water organisms are hypertonic (osmotic pressure of body fluids is higher than that of the surrounding water). Hence, fresh water animals must constantly expend more energy to keep water out (i.e., high osmotic pressure fluids contain more salts, the action being then to dilute this concentration with more water).

1 Generally, marine invertebrates are narrowly poikilosmotic, i.e., the salt concentration of the body fluids changes with that of the external medium. This has special significance in estuarine situations where salt concentrations of the water often vary considerably in short periods of time.

2 Marine bony fish (teleosts) have lower salt content internally than externally (hypotonic). In order to prevent dehydration, water is ingested and salts are excreted through special cells in the gills.

IV FACTORS AFFECTING THE DISTRIBUTION OF MARINE ORGANISMS

A Salinity - The concentration of salts is not the same everywhere in the sea; in the open ocean salinity is much less variable than in the ever changing estuary or coastal water. Organisms have different tolerances to salinity which limit their distribution. The distributions may be in large water masses, such as the Gulf Stream, Sargasso Sea, etc., or in bays and estuaries.

1 In general, animals in the estuarine environment are able to withstand large and rapid changes in salinity and temperature. These animals are classified as

a Euryhaline ("eury" meaning wide) - wide tolerance to salinity changes.

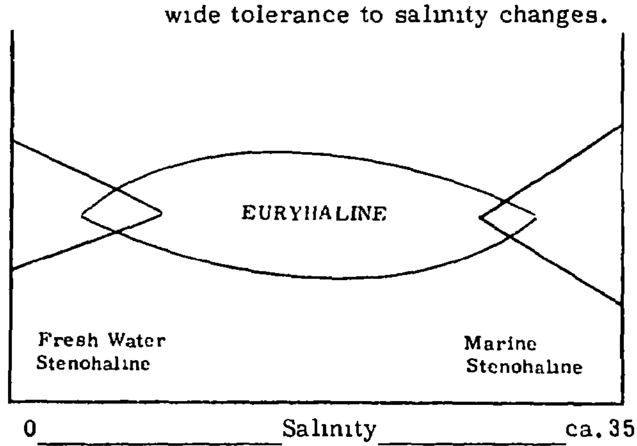
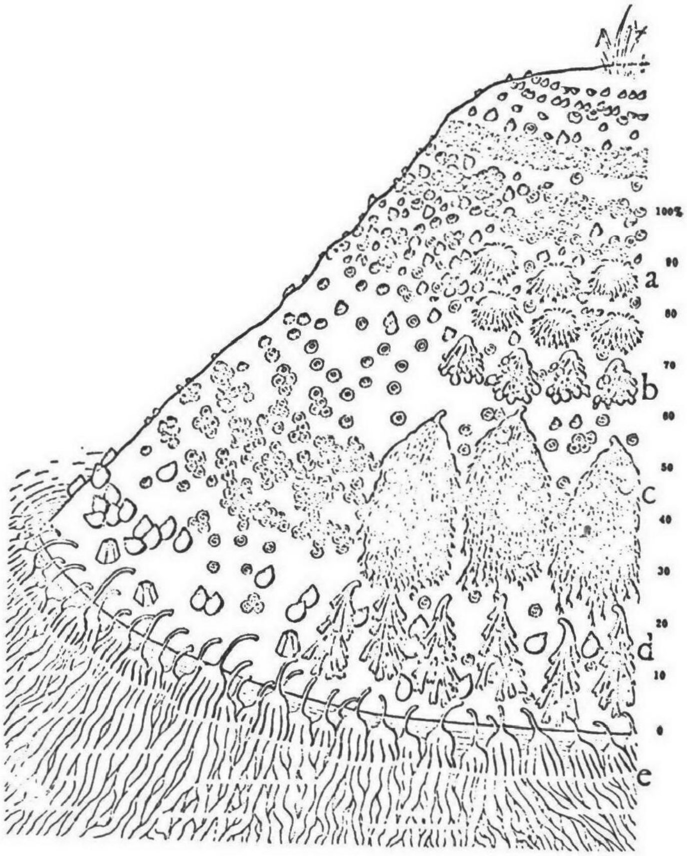


Figure 4. Salinity Tolerance of Organisms

b Eurythermal - wide tolerance to temperature changes.



SNAILS

- Littorina neritoides
- L. rudis
- L. obtusata
- L. littorea

BARNACLES

- Chthamalus stellatus
- Balanus balanoides
- B. perforatus

Figure 5

Zonation of plants, snails, and barnacles on a rocky shore. While this diagram is based on the situation on the southwest coast of England, the general idea of zonation may be applied to any temperate rocky ocean shore, though the species will differ. The gray zone consists largely of lichens. At the left is the zonation of rocks with exposure too extreme to support algae; at the right, on a less exposed situation, the animals are mostly obscured by the algae. Figures at the right hand margin refer to the percent of time that the zone is exposed to the air, i.e., the time that the tide is out. Three major zones can be recognized: the Littorina zone (above the gray zone); the Balanoid zone (between the gray zone and the laminarias); and the Laminaria zone. a. Pelvetia canaliculata; b. Fucus spiralis; c. Ascophyllum nodosum; d. Fucus serratus; e. Laminaria digitata. (Based on Stephenson)

2 In general, animals in river and oceanic environments cannot withstand large and rapid changes in salinity and temperature. These animals are classified as

a Stenohaline ("steno" meaning narrow) - narrow tolerance to salinity changes.

b Stenothermal - narrow tolerance to temperature changes.

3 Among euryhaline animals, those living in lowered salinities often have a smaller maximum size than those of the same species living in more saline waters. For example, the lamprey (Petromyzon marinus) attains a length of 30 - 36" in the sea, while in the Great Lakes the length is 18 - 24".

4 Usually the larvae of marine organisms are more sensitive to changes in salinity than are the adults This characteristic limits both the distribution and size of populations.

B Tides

Tidal fluctuation is a phenomenon unique to the seas (with minor exceptions). It is a twice daily rise and fall in the sea level caused by the complicated interaction of many factors including sun, moon, and the daily rotation of the earth. Tidal heights vary from day to day and place to place, and are often accentuated by local meteorological conditions. The rise and fall may range from a few inches or less to fifty feet or more.

V FACTORS AFFECTING THE PRODUCTIVITY OF THE MARINE ENVIRONMENT

The sea is in continuous circulation. Without circulation, nutrients of the ocean would eventually become a part of the bottom and biomass production would cease. Generally, in all oceans there exists a warm surface layer which overlies the colder water and forms a two-layer system of persistent stability. Nutrient concentration is usually greatest in the lower zone. Wherever a mixing or disturbance of these two layers occurs, biomass production is greatest. Factors causing this breakup are, therefore, of utmost importance concerning productivity.

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THE AQUATIC ENVIRONMENT

Part 5: Tidal Marshes

I INTRODUCTION: The Marsh and the Estuary

A "There is no other case in nature, save in the coral reefs, where the adjustment of organic relations to physical condition is seen in such a beautiful way as the balance between the growing marshes and the tidal streams by which they are at once nourished and worn away." (Shaler, 1886)

B Estuarine pollution studies are usually devoted to the dynamics of the circulating water, its chemical, physical, and biological parameters, bottom deposits, etc.

C It is easy to overlook the intimate relationwhips which exist between the bordering marshland, the moving waters, the tidal flats, subtidal deposition, and seston whether of local, oceanic, or riverine origin.

D The tidal marsh (some inland areas also have salt marshes) is generally considered to be the marginal areas of estuaries and coasts in the intertidal zone which are dominated by emergent vegetation. They generally extend inland to the farthest point reached by the spring tides, where they merge into freshwater swamps and marshes (Figure 1). They may range in width from nonexistent on rocky coasts to many kilometers.

II MARSH ORIGINS AND STRUCTURES

A In general, marsh substrates are high in organic content, relatively low in minerals and trace elements. The upper layers bound together with living roots called turf, underlaid by more compacted peat type material.

1 Rising or eroding coastlines may expose peat from ancient marsh growth to wave action which cuts into the soft peat rapidly (Figure 2). Such banks are likely to be cliff-like, and are often undercut. Chunks of peat are often found lying about on harder substrate below high tide line. If face of cliff is well above high water, overlying vegetation is likely to be typically terrestrial of the area. Marsh type vegetation is probably absent.

2 Low lying deltaic, or sinking coastlines, or those with low energy wave action are likely to have active marsh formation in progress (Figure 3). Sand dunes are also common in such areas (Figure 4). General coastal configuration is a factor.

a Rugged or precipitous coasts or slowly rising coasts, typically exhibit narrow shelves, sea cliffs, fjords, massive beaches, and relatively less marsh area (Figure 5). An Alaskan fjord subject to recent catastrophic subsidence and rapid deposition of glacial flour shows evidence of the recent encroachment of saline waters in the presence of recently buried trees and other terrestrial vegetation, exposure of layers of salt marsh peat along the edges of channels, and a poorly compacted young marsh turf developing at the new high water level (Figure 6).

b Low lying coastal plains tend to be fringed by barrier islands, broad estuaries and deltas, and broad associated marshlands (Figure 7, 14). Deep tidal channels fan out through innumerable branching and often interconnecting rivulets. The intervening grassy plains are essentially at mean high tide level.

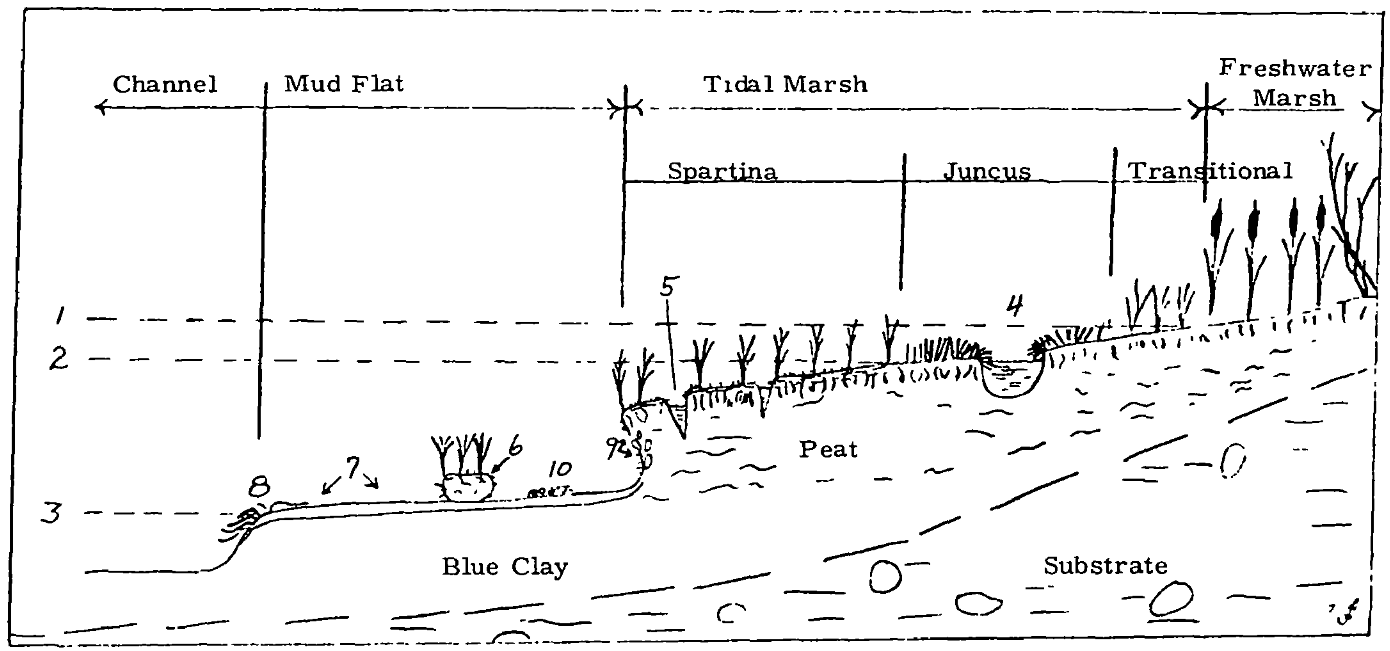

Figure 1 Zonation in a positive New England estuary 1. Spring tide level, 2. Mean high tide, 3. Mean low tide, 4. Bog hole, 5. Ice cleavage pool, 6. Chunk of Spartina turf deposited by ice, 7. Organic ooze with associated community, 8. eelgrass (Zostera), 9. Ribbed mussels (modiolus)-clam (mya) - mud snail (Nassa) community 10. Sea lettuce (Ulva)

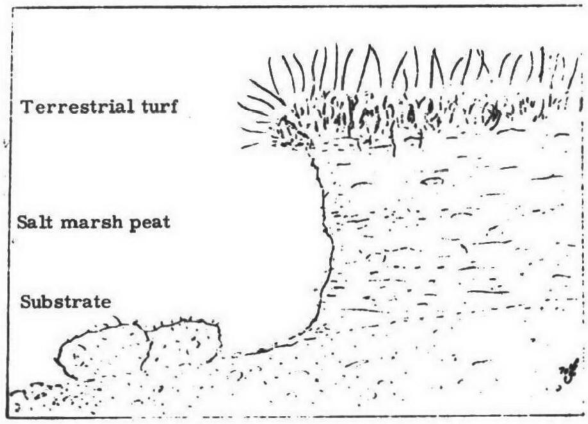

Figure 2. Diagrammatic section of eroding peat cliff

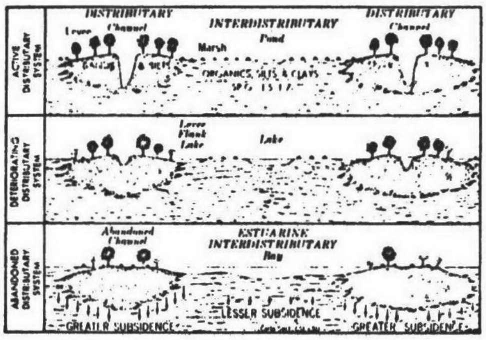

Figure 3. Effects of deltaic subsidence during distributary system abandonment

c Tropical and subtropical regions such as Florida, the Gulf Coast, and Central America, are frequented by mangrove swamps. This unique type of growth is able to establish itself in shallow water and move out into progressively deeper areas (Figure 8). The strong deeply embedded roots enable the mangrove to resist considerable wave action at times, and the tangle of roots quickly accumulates a deep layer of organic sediment. Mangroves are often considered to be effective as land builders. When fully developed, a mangrove swamp is an impenetrable thicket of roots over the tidal flat affording shelter to a sort of semi-aquatic organism such as various molluscs and crustaceans, and providing access from the nearby land to predaceous birds, reptiles and mammals.

Mangroves are not restricted to estuaries, but may develop out into shallow oceanic lagoons, or upstream into relatively fresh waters.

III PRODUCTIVITY OF MARSHES

A Measuring the productivity of grasslands is not easy, because grass is seldom used directly as such by man. It is thus usually expressed as production of meat, milk, or in the case of salt marshes, the total crop of animals that obtain food per unit of area. The primary producer in a tidal marsh is the marsh grass, but very little of it is used as grass. (Table 1)

The actual nutritional analysis of several marsh grasses as compared to dry land bay is shown in Table 2. A study of the yield of Juncus per square meter in a North Carolina marsh is shown in Figure 9.

B The actual utilization of marsh grass is accomplished primarily by its decomposition and ingestion by micro flora and fauna. A small quantity of seeds and solids is probably consumed directly by birds (Figure 10).

1 The quantity of micro invertebrates which thrive on this wealth of decaying marsh hay has not been estimated, nor has the actual production of small fishes such as the top minnows (Fundulus) which swarm in at high tide, or the mud snails (Nassa) and others. Many forms of oceanic life migrate into the estuaries, especially the marsh areas, for important portions of their life histories as has been mentioned elsewhere (Figure 11).

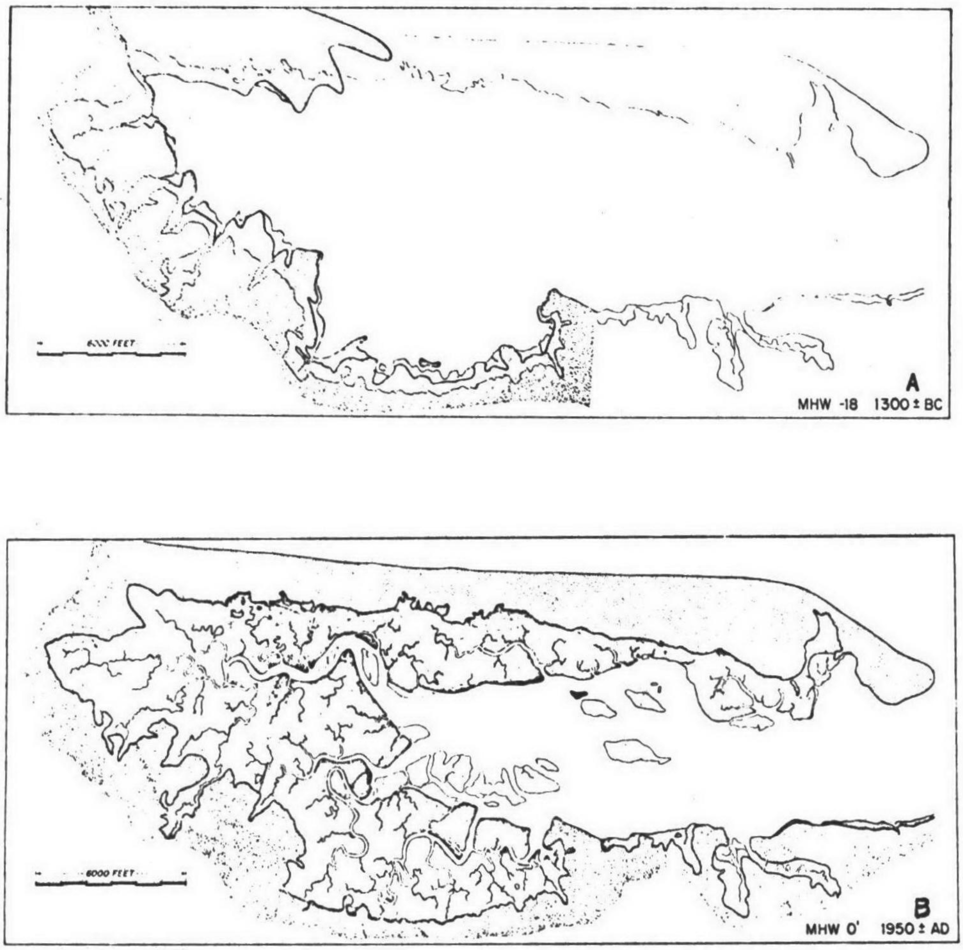

Figure 4

Development of a Massachusetts Marsh since 1300 BC, involving an 18 foot rise in water level. Shaded area indicates sand dunes. Note meandering marsh tidal drainage. A: 1300 BC, B: 1950 AD.

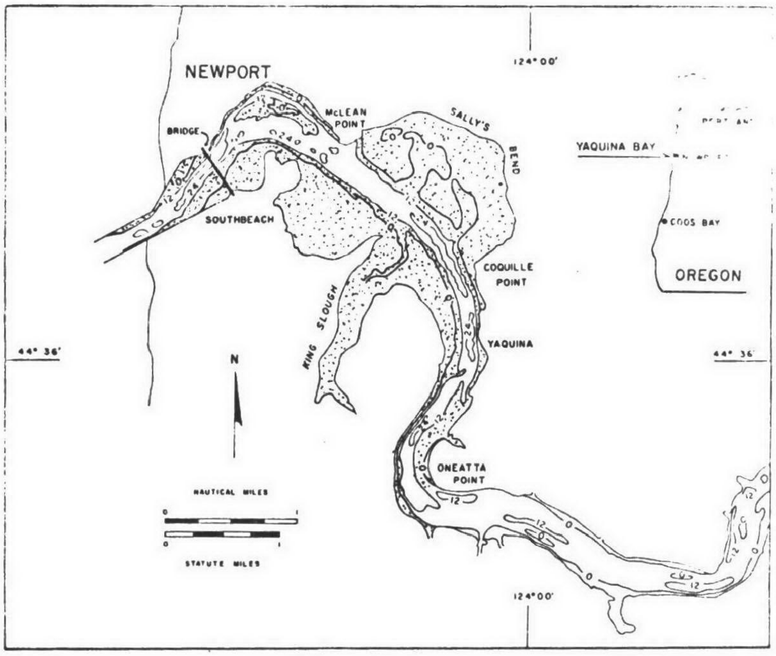

Figure 5. A River Mouth on a Slowly Rising Coast. Note absence of deltaic development and relatively little marshland, although mud flats stippled are extensive.

2 An indirect approach in Rhode Island revealed in a single August day on a relatively small marsh area, between 700 and 1000 wild birds of 12 species, ranging from 100 least sandpipers to uncountable numbers of seagulls. One food requirement estimate for three-pound poultry in the confined inactivity of a poultry yard is approximately one ounce per pound of bird per day. One-hundred (100) black bellied plovers at approximately ten (10) ounces each would weigh on the order of sixty (60) pounds. At the same rate of food consumption, this would indicate nearly four (4) pounds of food required for this species alone. The much greater activity of the wild birds would obviously greatly increase their food requirements, as would their relatively smaller size.

Considering the range of foods consumed, the sizes of the birds, and the fact that at certain seasons, thousands of migrating ducks and others pause to feed here, the enormous productivity of such a marsh can be better understood.

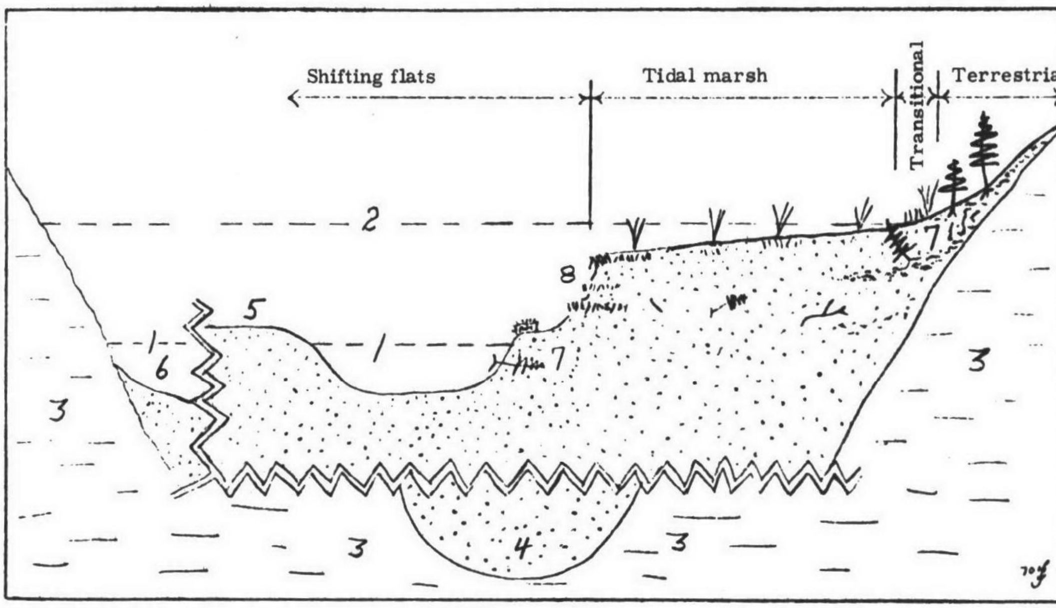

Figure 6. Some general relationships in a northern fjord with a rising water level. 1. mean low water, 2. maximum high tide, 3. Bedrock, 4. Glacial flour to depths in excess of 400 meters, 5. Shifting flats and channels, 6. Channel against bedrock, 7. Buried terrestrial vegetation, 8. Outcroppings of salt marsh peat.

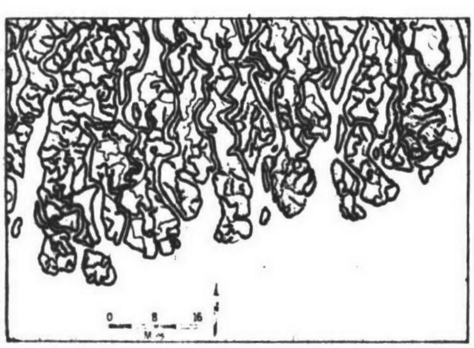

Figure 7. A Coastal Plain Marsh in India subject to a high tidal range.

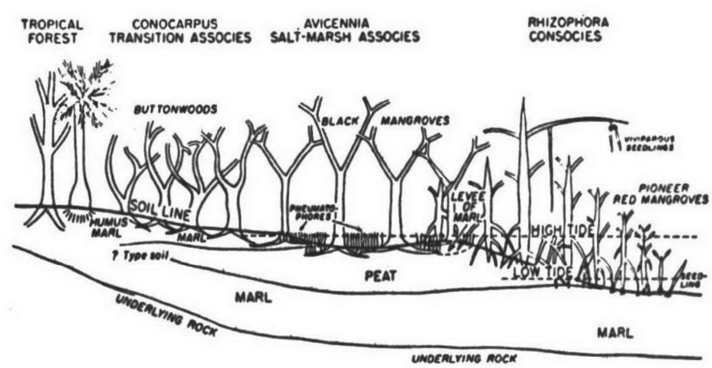

Figure 8. Diagrammatic transect of a mangrove swamp showing transition from marine to terrestrial habitat.

TABLE 1. General Orders of Magnitude of Gross Primary Productivity in Terms of Dry Weight of Organic Matter Fixed Annually

Ecosystem	$gms/M^2/year$ (grams/square meters/year)	lbs/acre/year
Land deserts, deep oceans	Tens	Hundreds
Grasslands, forests, eutrophic lakes, ordinary agriculture	Hundreds	Thousands
Estuaries, deltas, coral reefs, intensive agriculture (sugar cane, rice)	Thousands	Ten-thousands

TABLE 2. Analyses of Some Tidal Marsh Grasses

T/A Dry Wt	Percentage Composition Protein	Fat	Fiber	Water	Ash	N-free Extract
Distichlis spicata (pure stand, dry)						
2.8	5.3	1.7	32.4	8.2	6.7	45.5
Short *Spartina alterniflora* and *Salicornia europaea* (in standing water)						
1.2	7.7	2.5	31.1	8.8	12.0	37.7
Spartina alterniflora (tall, pure stand in standing water)						
3.5	7.6	2.0	29.0	8.3	15.5	37.3
Spartina patens (pure stand, dry)						
3.2	6.0	2.2	30.0	8.1	9.0	44.5
Spartina alterniflora and *Spartina patens* (mixed stand, wet)						
3.4	6.8	1.9	29.8	8.1	10.4	42.8
Spartina alterniflora (short, wet)						
2.2	8.8	2.4	30.4	8.7	13.3	36.3
Comparable Analyses for Hay						
1st cut	6.0	2.0	36.2	6.7	4.2	44.9
2nd cut	13.0	3.7	28.5	10.4	5.9	40.5

Analyses performed by Roland W. Gilbert, Department of Agricultural Chemistry, U.R.I

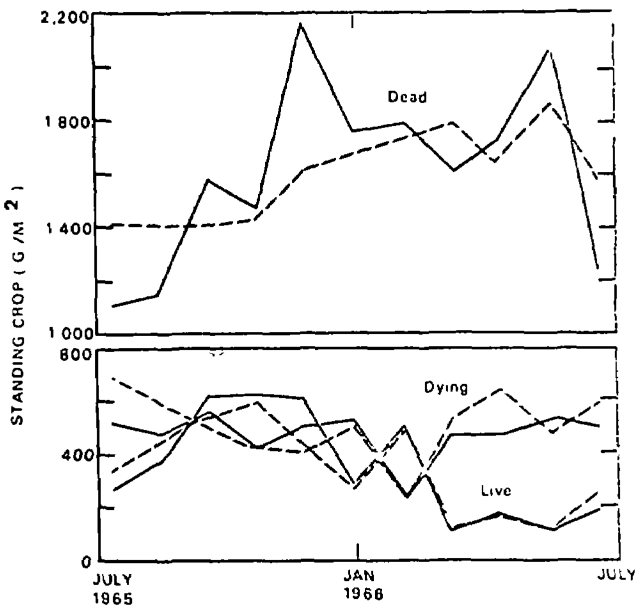

Figure 9. Standing crop of Juncus. Solid line represents observed values, broken line represents seasonal cycle calculated on the basis of an assumed constant total biomass.

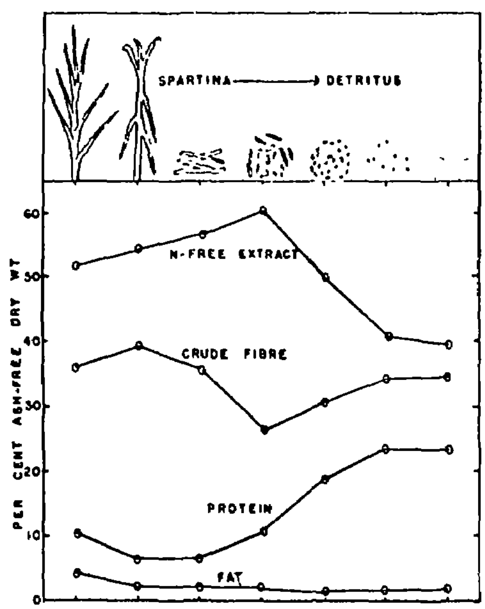

Figure 10. The nutritive composition of successive stages of decomposition of Spartina marsh grass, showing increase in protein and decrease in carbohydrate with increasing age and decreasing size of detritus particles.

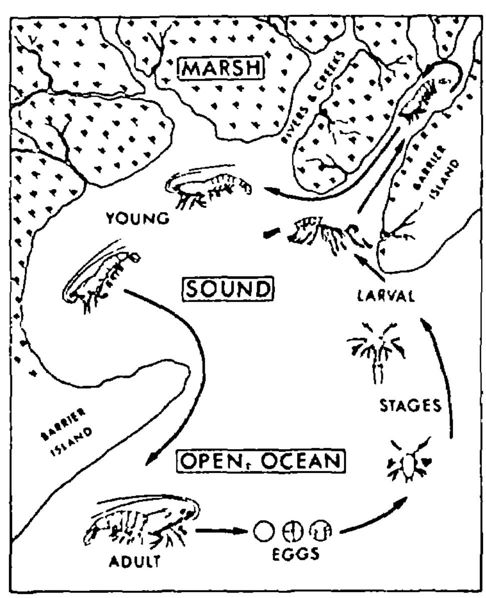

Figure 11. Diagram of the life cycle of white shrimp (after Anderson and Lunz 1965).

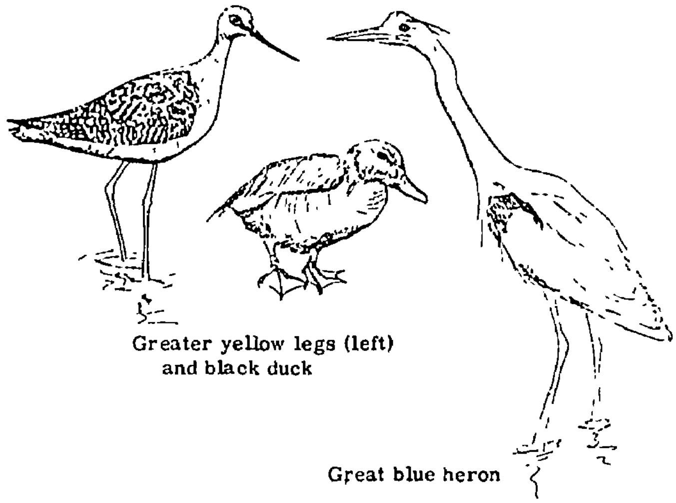

Figure 12. Some Common Marsh Birds

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I INTRODUCTION

A All aquatic organisms do not react uniformly to the various chemical, physical and biological features in their environment Through normal evolutionary processes various organisms have become adapted to certain combinations of environmental conditions. The successful development and maintenance of a population or community depend upon harmonious ecological balance between environmental conditions and tolerance of the organisms to variations in one or more of these conditions.

B A factor whose presence or absence exerts some restraining influence upon a population through incompatibility with species requirements or tolerance is said to be a limiting factor. The principle of limiting factors is one of the major aspects of the environmental control of aquatic organisms (Figure 1).

II PRINCIPLE OF LIMITING FACTORS

This principle rests essentially upon two basic concepts. One of these relates organisms to the environmental supply of materials essential for their growth and development The second pertains to the tolerance which organisms exhibit toward environmental conditions.

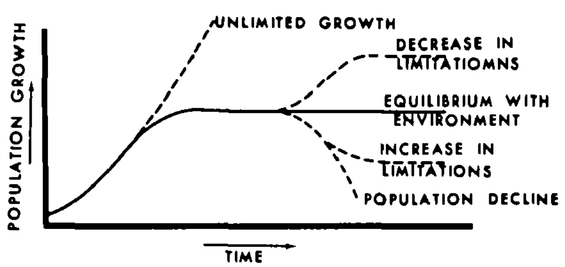

Figure 1 The relationships of limiting factors to population growth and development

A Liebig's Law of the Minimum enunciates the first basic concept In order for an organism to inhabit a particular environment, specified levels of the materials necessary for growth and development (nutrients, respiratory gases, etc.) must be present. If one of these materials is absent from the environment or present in minimal quantities, a given species will only survive in limited numbers, if at all (Figure 2).

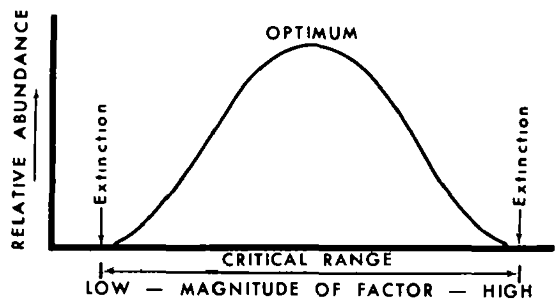

Figure 2. Relationships of environmental factors and the abundance of organisms.

1 The subsidiary principle of factor interaction states that high concentration or availability of some substance, or the action of some factor in the environment, may modify utilization of the minimum one. For example

a The uptake of phosphorus by the algae Nitzchia closterium is influenced by the relative quantities of nitrate and phosphate in the environment; however, nitrate utilization appears to be unaffected by the phosphate (Reid, 1961).

b The assimilation of some algae is closely related to temperature.

c The rate of oxygen utilization by fish may be affected by many other substances or factors in the environment.

d Where strontium is abundant, mollusks are able to substitute it, to a partial extent, for calcium in their shells (Odum, 1959).

2 If a material is present in large amounts, but only a small amount is available for use by the organism, the amount available and not the total amount present determines whether or not the particular material is limiting (calcium in the form of $CaCO_3$).

B Shelford pointed out in his Law of Tolerance that there are maximum as well as minimum values of most environmental factors which can be tolerated Absence or failure of an organism can be controlled by the deficiency or excess of any factor which may approach the limits of tolerance for that organism (Figure 3).

Minimum Limit of Toleration		Range of Optimum of Factors	Maximum Limit of Toleration	
Absent	Decreasing Abundance	Greatest Abundance	Decreasing Abundance	Absent

Figure 3. Shelford's Law of Tolerance.

1 Organisms have an ecological minimum and maximum for each environmental factor with a range in between called the critical range which represents the range of tolerance (Figure 2). The actual range thru which an organism can grow, develop and reproduce normally is usually much smaller than its total range of tolerance.

2 Purely deleterious factors (heavy metals, pesticides, etc.) have a maximum tolerable value, but no optimum (Figure 4).

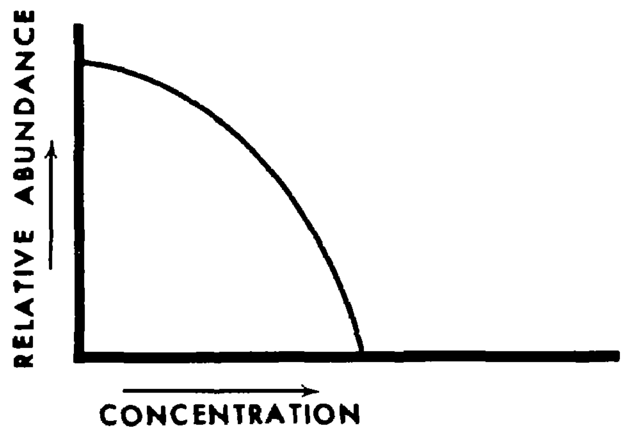

Figure 4. Relationship of purely harmful factors and the abundance of organisms.

3 Tolerance to environmental factors varies widely among aquatic organisms.

a A species may exhibit a wide range of tolerance toward one factor and a narrow range toward another. Trout, for instance, have a wide range of tolerance for salinity and a narrow range for temperature.

b All stages in the life history of an organism do not necessarily have the same ranges of tolerance. The period of reproduction is a critical time in the life cycle of most organisms.

c The range of tolerance toward one factor may be modified by another factor. The toxicity of most substances increases as the temperature increases.

d The range of tolerance toward a given factor may vary geographically within the same species. Organisms that adjust to local conditions are called ecotypes.

e The range of tolerance toward a given factor may vary seasonally. In general organisms tend to be more sensitive to environmental changes in summer than in other seasons. This is primarily due to the higher summer temperatures.

4 A wide range of distribution of a species is usually the result of a wide range of tolerances. Organisms with a wide range of tolerance for all factors are likely to be the most widely distributed, although their growth rate may vary greatly. A one-year old carp, for instance, may vary in size from less than an ounce to more than a pound depending on the habitat.

5 To express the relative degree of tolerance for a particular environmental factor the prefix eury (wide) or steno (narrow) is added to a term for that feature (Figure 5).

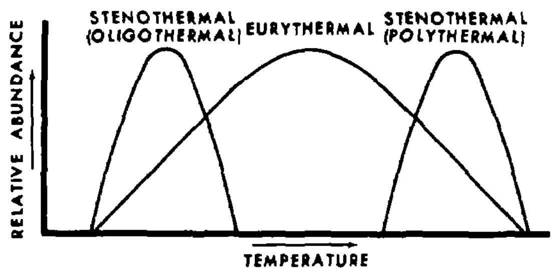

Figure 5. Comparison of relative limits of tolerance of stenothermal and eurythermal organisms.

C The law of the minimum as it pertains to factors affecting metabolism, and the law of tolerance as it relates to density and distribution, can be combined to form a broad principle of limiting factors.

1 The abundance, distribution, activity and growth of a population are determined by a combination of factors, any one of which may through scarcity or overabundance be limiting.

2 The artificial introduction of various substances into the environment tends to eliminate limiting minimums for some species and create intolerable maximums for others.

3 The biological productivity of any body of water is the end result of interaction of the organisms present with the surrounding environment.

III VALUE AND USE OF THE PRINCIPLE OF LIMITING FACTORS

A The organism-environment relationship is apt to be so complex that not all factors are of equal importance in a given situation; some links of the chain guiding the organism are weaker than others. Understanding the broad principle of limiting factors and the subsidiary principles involved make the task of ferreting out the weak link in a given situation much easier and possibly less time consuming and expensive.

1 If an organism has a wide range of tolerance for a factor which is relatively constant in the environment that factor is not likely to be limiting. The factor cannot be completely eliminated from consideration, however, because of factor interaction.

2 If an organism is known to have narrow limits of tolerance for a factor which is also variable in the environment, that factor merits careful study since it might be limiting.

B Because of the complexity of the aquatic environment, it is not always easy to isolate the factor in the environment that is limiting a particular population. Premature conclusions may result from limited observations of a particular situations. Many important factors may be overlooked unless a sufficiently long period of time is covered to permit the factors to fluctuate within their ranges of possible variation. Much time and money may be wasted on control measures without the real limiting factor ever being discovered or the situation being improved.

C Knowledge of the principle of limiting factors may be used to limit the number of parameters that need to be measured or observed for a particular study. Not all of the numerous physical, chemical and biological parameters need to be measured or observed for each study undertaken. The aims of a pollution survey are not to make and observe long lists of possible limiting factors but to discover which factors are significant, how they bring about their effects, the source or sources of the problem, and what control measures should be taken.

D Specific factors in the aquatic environment determine rather precisely what kinds of organisms will be present in a particular area. Therefore, organisms present or absent can be used to indicate environmental conditions. The diversity of organisms provides a better indication of environmental conditions than does any single species. Strong physio-chemical limiting factors tend to reduce the diversity within a community; more tolerant species are then able to undergo population growth.

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This outline was prepared by John E. Matthews, Aquatic Biologist, Robert S. Kerr Water Research Center, Ada, Oklahoma.

THE SYSTEM OF BIOLOGICAL CLASSIFICATION

I INTRODUCTION

There are few major groups of organisms that are either exclusively terrestrial or generally aquatic. The following remarks apply to both, however, primary attention will be directed to aquatic types.

II CLASSIFICATION

One of the first questions usually posed about an organism seen for the first time is "what is it?" usually meaning, "what is its name?" The naming or classification of biological organisms is a science in itself (taxonomy). Some of the principles involved need to be understood by anyone working with organisms however.

A Names are the "key number", "code designation", or "file references" which we must have to find information about an unknown organism.

B Why are they so long and why must they be in Latin and Greek? File references in large systems have to be long in order to designate the many divisions and sub-divisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have as many as a million books to classify).

C Common names are rarely available for most invertebrates and algae. Exceptions to this are common among the molluscs, many of which have common names which are fairly standard for the same species throughout its range. This may be due to their status as a commercial harvest or to the activities of devoted groups of amateur collectors. Certain scientific societies have also assigned "official" common names to particular species, for example, aquatic weeds - American Weed Society, fish - American Fisheries Society, amphibians (salamanders and frogs) - American Society of Ichthyologists and Herpetologists.

D The system of biological nomenclature is regulated by international congresses.

1 It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the <u>species</u>.

2 The taxa (categories) employed are as follows

The <u>species</u> is the foundation (plural species)

Similar species are grouped into <u>genera</u> (singular· genus).

Similar genera are grouped into <u>families</u>.

Similar families are grouped into <u>orders</u>.

Similar orders are grouped into <u>classes</u>.

Similar classes are grouped into <u>phyla</u> (phylum).

Similar phyla are grouped into <u>kingdoms</u>.

Other categories such as sub-species, variety, strain, division, tribe, etc. are employed in special circumstances.

D The <u>scientific name</u> of an organism is its generic name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names).

1 The generic (genus) name is always capitalized and the species name written with a small letter. They

should also be underlined or printed in italics when used in a technical sense. For example:

Homo sapiens - (=sentiens) modern man

Homo heidelbergensis - heidelberg man

Homo neanderthalis - neanderthal man

Oncorhynchus gorbuscha - pink salmon

Oncorhynchus kisutch - coho salmon

Oncorhynchus tshawytscha - chinook salmon

2 Common names do not exist for most of the smaller and less familiar organisms. For example, if we wish to refer to members of the genus Gomphonema (a diatom) we must simply use the generic name, and:

Gomphonema olivaceum

Gomphonema parvulum

Gomphonema abbreviatum

three distinct species which have different significances to algologists interpreting water quality

3 A complete list of the various categories to which an organism belongs is known as its "classification" For example, the classification of a type of diatom and a midge larva or "bloodworm" are shown side by side below Their scientific names are Gomphonema olivaceum and Chironomus riparius.

a Examples of the Classification of an animal and a plant:

Kingdom	Plantae	Animalia
Phylum	Chrysophyta	Arthropoda
Class	Bacillariophyceae	Insecta
Order	Pennales	Diptera
Family	Gomphonemaceae	Chironomida
Genus	Gomphonema	Chironomus
Species	olivaceum	riparius

b These seven basic levels of organization are often not enough for the complete designation of one species among thousands, however, and so additional echelons of terms are provided by grouping the various categories into "super..." groups and subdividing them into "sub..." groups as

Superorder, Order, Suborder, etc., Still other category names such as "tribe", "division","variety", "race", "section", etc., are used on occasion.

c Additional accuracy is gained by citing the name of the authority who first described a species (and the date) immediately following the species name. Authors are also often cited for genera or other groups.

d A more complete classification of the above midge, follows·

Kingdom Animalia

Superphylum Annelid

Phylum Arthropoda

Class Insecta

Order Diptera

Suborder Nematocera

Family Chironomidae

Subfamily Chironominae

Tribe Chironomini

Genus Chironomus

Species riparius Meigen 1804

e It should be emphasized that since all categories above the species level are essentially human concepts, there is often divergence of opinion in regard to how certain organisms should be grouped. Changes result as knowledge grows.

f The most appropriate or correct names too are subject to change. The species itself, however, as an entity in nature, is relatively timeless and so does not change to man's eye.

This outline was prepared by H. W. Jackson, Chief Biologist and R. M. Sinclair, Aquatic Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

AQUATIC ORGANISMS OF SIGNIFICANCE IN POLLUTION SURVEYS

I INTRODUCTION

A Any organism encountered in a survey is of significance Our problem is thus not to determine which are of significance but rather to decide "what is the significance of each?"

B The first step in interpretation is recognition "The first exercise in ecology is systematics "

C Recognition implies identification and an understanding of general relationships (systematics) The following outline will thus review the general relationships of living (as contrasted to fossil) organisms and briefly describe the various types

II THE GENERAL RELATIONSHIPS OF LIVING ORGANISMS

A Living organisms have long been grouped into two kingdoms· plant and animal Modern developments, however, have made this simple pattern technically untenable It has become evident that there are as great and fundamental differences between certain other groups and these (two), as there are between the traditional "plant" and "animal." The accompanying chart consequently shows the fungi as a third "kingdom "

B The three groups are essentially defined as follows on the basis of their nutritional mechanisms (see figure

1 Plantae· photosynthetic, synthetizing their own organic substance from inorganic minerals Ecologically known as PRODUCERS.

2 Animalia ingest and digest solid particles of organic food material Ecologically known as CONSUMERS.

3 Fungi extracellular digestion (enzymes secreted externally) Food material then taken in through cell membrane where it is metabolized and reduced to the mineral condition Ecologically known as REDUCERS.

C Each of these groups includes simple, single-celled representatives, persisting at lower levels on the evolutionary stems of the higher organisms.

1 These groups span the gaps between the higher kingdoms with a multitude of transitional forms They are collectively called the PROTISTA.

2 Within the protista, two principal sub-groups can be defined on the basis of relative complexity of structure

a The bacteria and blue-green algae, lacking a nuclear membrane may be considered as the lower protista (or Monera)

b The single-celled algae and protozoa are best referred to as the higher protista

D Distributed throughout these groups will be found most of the traditional "phyla" of classic biology.

III PLANTS

A The vascular plants are usually larger and possess roots, stems and leaves.

1 Some types emerge above the surface (emersed).

2 Submersed types typically do not extend to the surface.

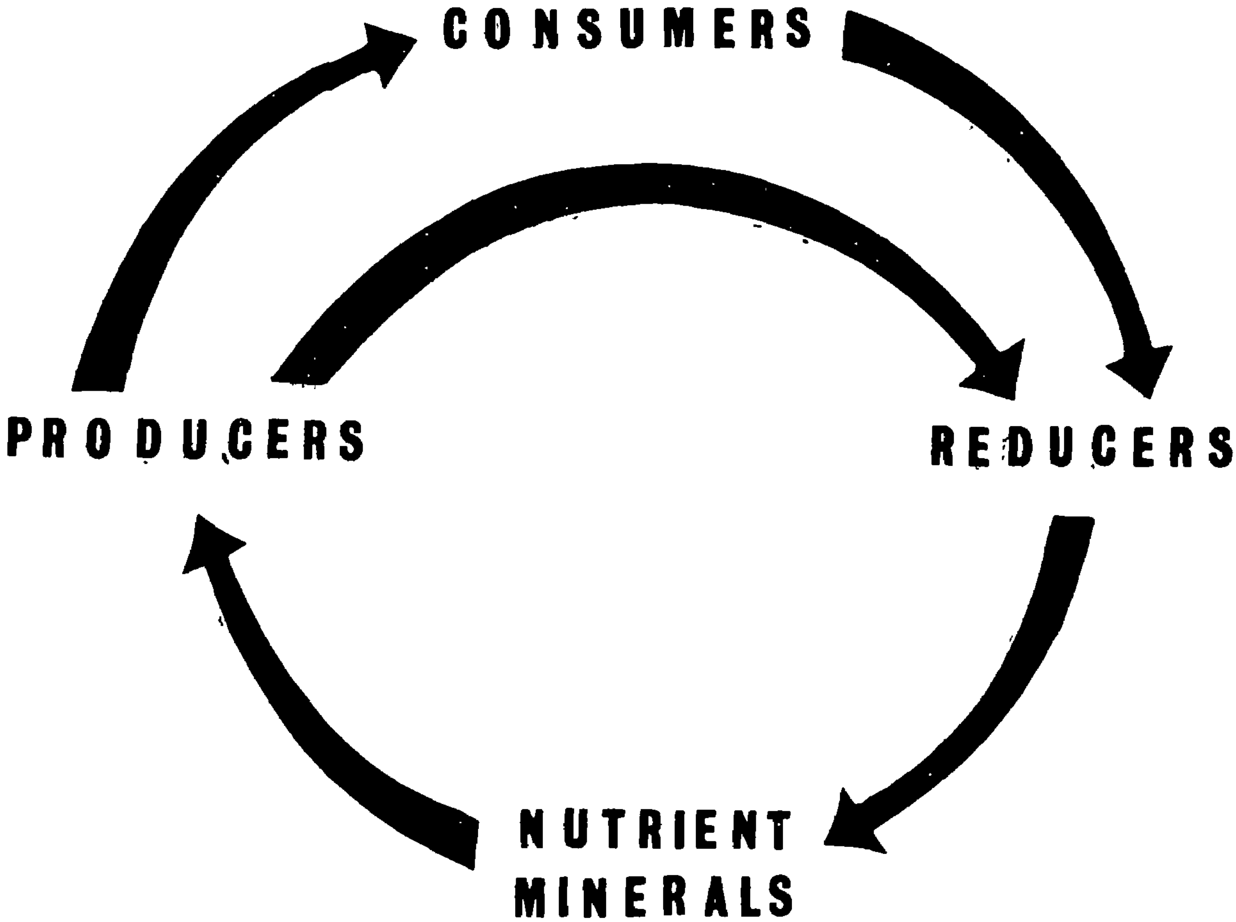

Figure 1. BASIC CYCLES OF LIFE

3 Floating types may be rooted or free-floating.

B Algae generally smaller, more delicate, less complex in structure, possess chlorophyll like other green plants. For convenience the following artificial grouping is used in sanitary science.

1 "Blue-green algae" are typically small and lack an organized nucleus, pigments are dissolved in cell sap Structure very simple.

2 "Pigmented flagellates" possess nuclei, chloroplasts, flagellae and a red eye spot This is an artificial group containing several remotely related organisms, may be green, red, brown, etc.

3 "Diatoms" have "pillbox" structure of SiO_2 - may move. Extremely common Many minute in size, but colonial forms may produce hair-like filaments Golden brown in color

4 "Non-motile green algae" have no locomotor structure or ability in mature condition Another artificial group

a Unicellular representatives may be extremely small

b Multicellular forms may produce great floating mats of material

IV FUNGI

Lack chlorophyll and consequently most are dependent on other organisms They secrete extracellular enzymes and reduce complex organic material to simple compounds which they can absorb directly through the cell wall.

A Schizomycetes or bacteria are typically very small and do not have an organized nucleus

1 Autotrophic bacteria utilize basic food materials from inorganic substrates. They may be photo-synthetic or chemosynthetic.

2 Heterotrophic bacteria are most common. They require organic material on which to feed.

B "True fungi" usually exhibit hyphae as the basis of structure

V ANIMALS

A Lack chlorophyll and consequently feed on or consume other organisms. Typically ingest and digest their food.

B The Animal Phyla

1 PROTOZOA are single celled organisms, many resembling algae but lacking chlorophyll (cf illustration in "Oxygen" lecture)

2 PORIFERA are the sponges, both marine and freshwater representatives.

3 CNIDARIA (= COELENTERATA) include corals, marine and fresh-water jelly fishes, marine and freshwater hydroids.

4 PLATYHELMINTHES are the flat worms such as tape worms, flukes and Planaria.

5 NEMATHELMINTHES are the round worms and include both free-living forms and many dangerous parasites.

6 ROTIFERS are multicellular microscopic predators

7 BRYOZOA are small colonial sessile forms, marine or freshwater

8 MOLLUSCA include snails and slugs, clams, mussels and oysters, squids and octopi

9 BRACHIOPODS are bivalved marine organisms usually observed as fossils

10 ANNELIDS are the segmented worms such as earthworms, sludge worms and many marine species

11 ECHINODERMS include starfish, sea urchins and brittle stars. They are exclusively marine.

12 CTENOPHORES, or comb jellies, are delicate jelly-like marine organisms.

13 ARTHROPODA, the largest of all animal phyla. They have jointed appendages and a chitinous exoskelton.

a CRUSTACEA are divided into a cephalothorax and abdomen, and have many pairs of appendages, including paired antennae.

1) CLADOCERA include Daphnia a common freshwater microcrustacean, swim by means of branched antennae.

2) ANOSTRACA (=PHYLLOPODS) are the fairy shrimps, given to eruptive appearances in temporary pools.

3) COPEPODES are marine and freshwater microcrustacea--swim by means of unbranched antennae.

4) OSTRACODS are like microscopic "clams with legs."

5) ISOPODS are dorsoventrally compressed, called sowbugs. Terrestrial and aquatic, marine and freshwater.

6) AMPHIPODA - known as scuds, laterally compressed. Marine and freshwater.

7) DECAPODA - crabs, shrimp, crayfish, lobsters, etc. Marine and freshwater.

b INSECTA - body divided into head, thorax and abdomen, 3 pairs of legs, adults typically with 2 pairs of wings and one pair of antennae. No common marine species. Nine of the twenty-odd orders include species with freshwater-inhabiting stages in their life history as follows

1) DIPTERA - two-winged flies

2) COLEOPTERA - beetles

3) EPHEMEROPTERA - may flies

4) TRICHOPTERA - caddis flies

5) PLECOPTERA - stone flies

6) ODONATA - dragon flies and damsel flies

7) NEUROPTERA - alder flies, Dobson flies and fish flies

8) HEMIPTERA - true bugs, sucking insects such as water striders, electric light bugs and water boatman

9) LEPIDOPTERA - butterflies and moths, includes a few freshwater moths

c ARACHINIDA - body divided into cephalothorax and abdomen, 4 pairs of legs - spiders, scorpions, ticks and mites. Few aquatic representatives except for the freshwater mites and tardigrades.

C CHORDATA

1 PROTOCHORDATES - primitive marine forms such as acorn worms, sea squirts and lancelets

2 VERTEBRATES - all animals which have a backbone

a PISCES or fishes· including such forms as sharks and rays, lampreys, and higher fishes, both marine and freshwater

b AMPHIBIA - frogs, toads, and salamanders - marine species rare

c REPTILA - snakes, lizards and turtles

d MAMMALS - whales and other warm-blooded vertebrates with hair

e AVES - birds - warm-blooded vertebrates with feathers

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Whittaker, R. H. New Concepts of Kingdoms of Organisms. Science 163:150-160. 1969.

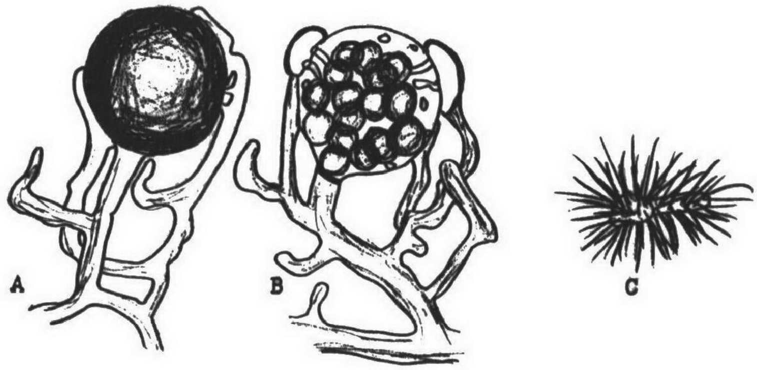
This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

FUNGI

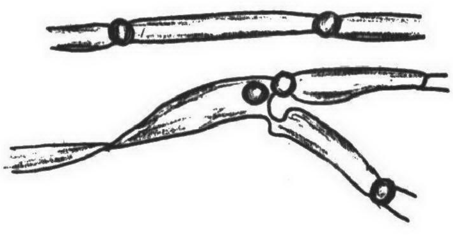
3/4

Schizomycetes - Bacteria, free living representatives

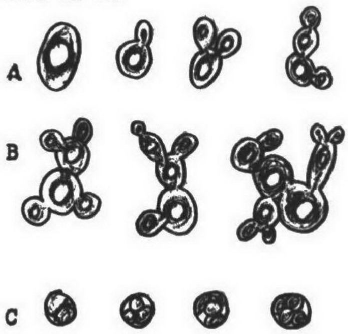
<u>Aerobacter aerogenes</u> <u>Rhizobium radicicola</u> <u>Azotobacter</u>



Phycomycetes - <u>Saprolegnia</u>; A, detail of immature reproductive stages; B, mature oogonium and antheridia, with eggs and fertilization tubes; C, dead tadpole with growth of S.



Phycomycete - <u>Leptomitus</u>, this genus includes pollution tollerant species.



Ascomycete - <u>Saccharomyces</u>, a yeast including poll. tollerant species. A, single cell; B, budding; C, ascospore formation.

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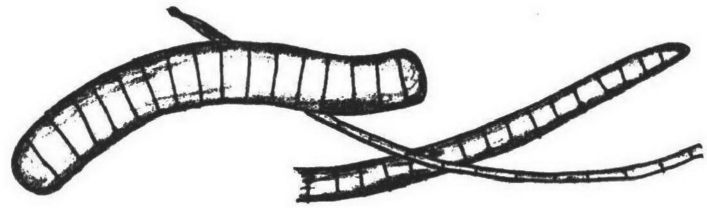
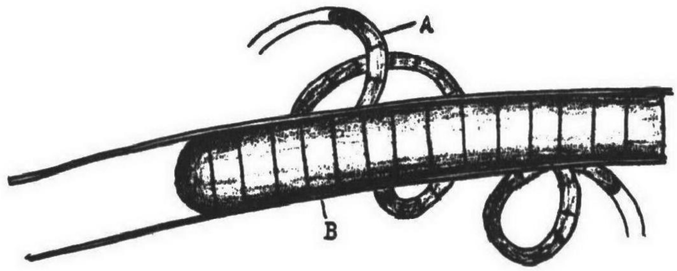
PLATE I

BLUE GREEN ALGAE

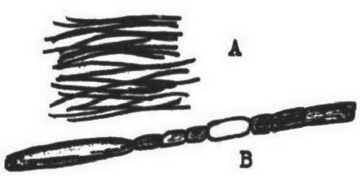
3/4



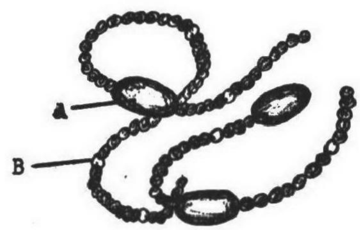
Oscillatoria spp., filaments (trichomes) range from .6 to over 60μ in diameter. Ubiquitous, pollution tollerant.



Lyngbia spp.. similar to Oscillatoria but has a sheath.
A, Lyngbia contorta, reported to be generally intollerant of pollution; B, L. birgei.



Aphanizomenon flos-aquae
A, colony; B, filament



Anabaena flos-aquae
A, akinete; B, heterocyst

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PLATE II

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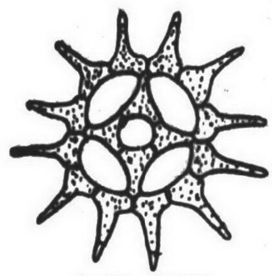
NON-MOTILE GREEN ALGAE: COCCOID (CHLOROPHYCEAE)



Pediastrum

Species of the Genus Scenedesmus

S. caudatus

S. abundans

S. dimorphus

Desmids

Closterium

Staurastrum

Cosmarium

PLATE III

11/3 NON-MOTILE GREEN ALGAE: FILAMENTOUS

(Chlorophyceae)

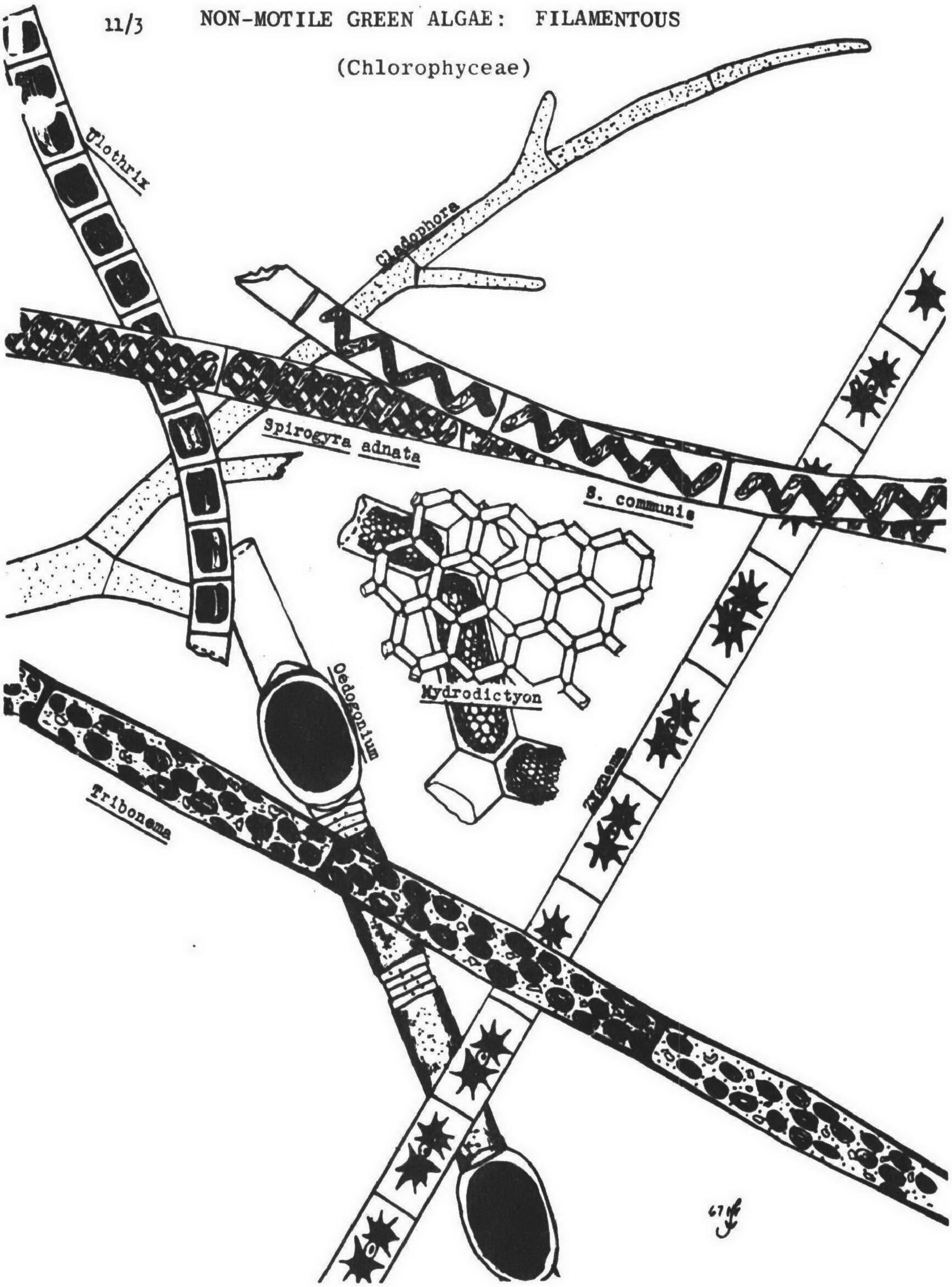


PLATE IV

PIGMENTED FLAGELLATES

3/4

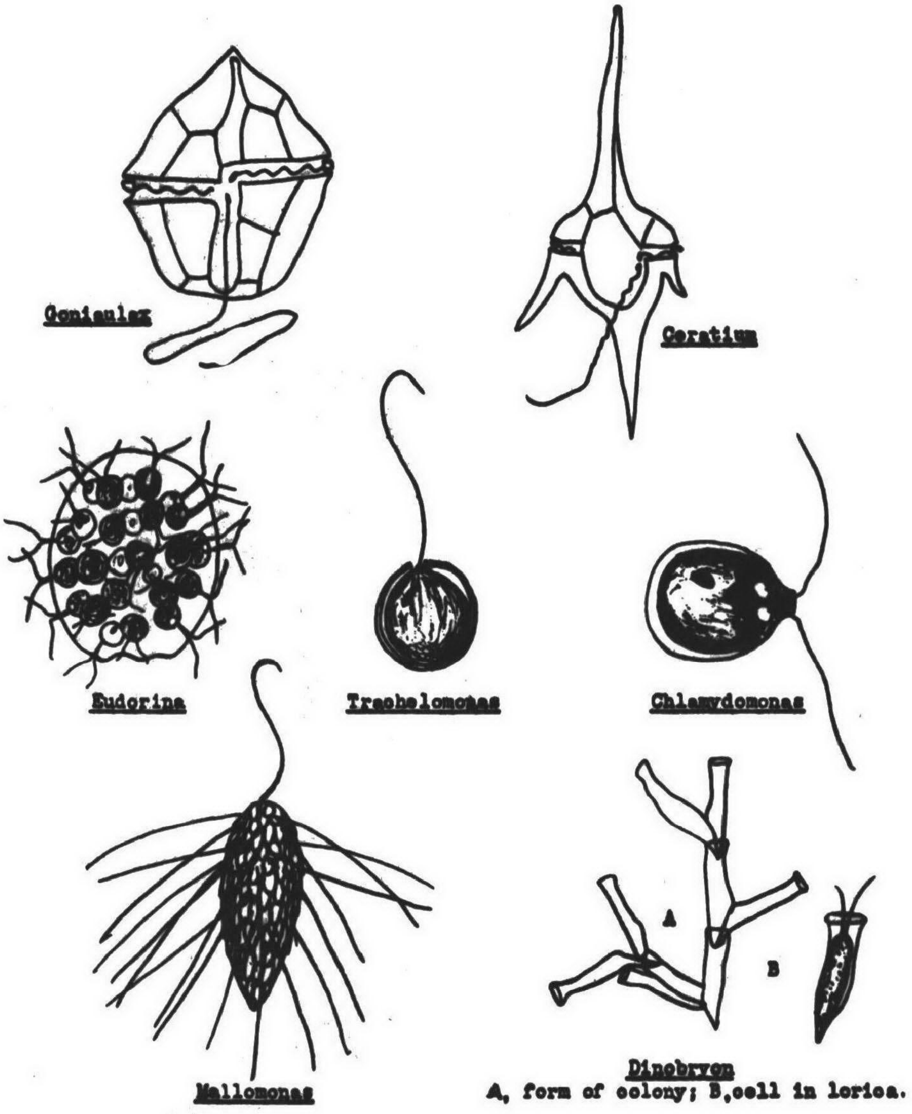


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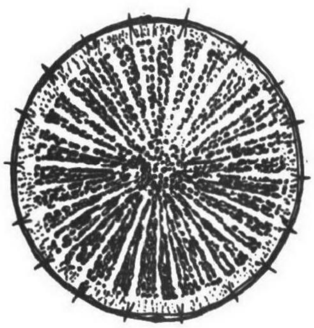
PLATE V

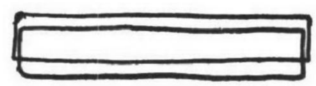
DIATOMS

3/4



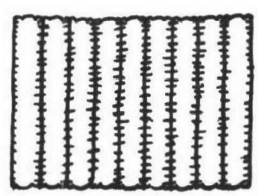
Valve views

Girdle views, stylized to show basic diatom structure.

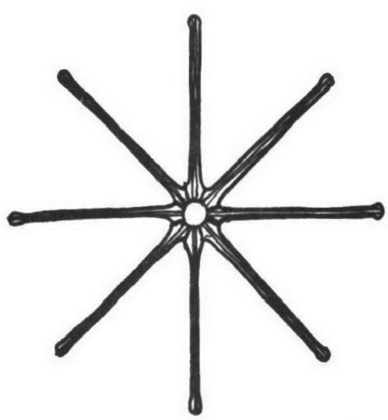


A discoid or central diatom such as <u>Stephanodiscus</u>

A pennate or navicular diatom such as <u>Fragillaria</u>



A colony of <u>Fragillaria</u> (girdle views)



A colony of <u>Asterionella</u> (girdle views)

<u>Gomphonema</u>
A, valve view; B, girdle view.

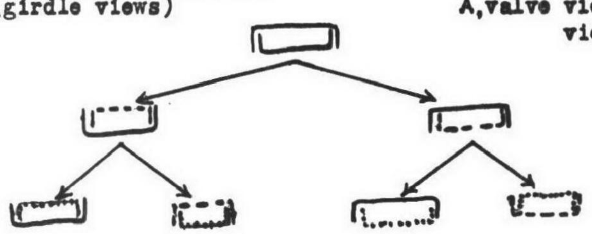


Diagram showing progressive diminution in the size of certain frustules through successive cell generations of a diatom.

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PLATE VI

3/4

FREE LIVING PROTOZOA

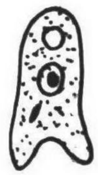
I. Flagellated Protozoa, Class Mastigophora

Anthophysis
Pollution tolerant
6 μ

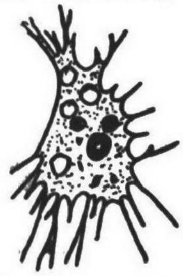
Bodo
Pollution tolerant
19 μ

Colony of Poteriodendron
Pollution tolerant , 35 μ

II. Ameboid Protozoa, Class Sarcodina



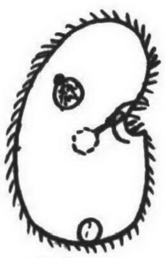
Dimastigamoeba
Pollution tolerant
10-50 μ



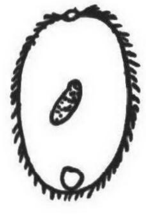
Nuclearia, reported to be intolerant of pollution, 45 μ.

Difflugia
Pollution tolerant
60-500 μ

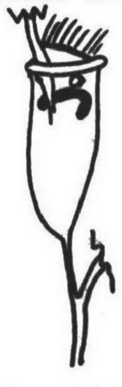
III. Ciliated Protozoa, Class Ciliophora



Colpoda
Pollution tolerant
20-120 μ



Holophrya, reported to be intolerant of pollution, 35 μ



Epistylis, pollution tolerant Colonies often macroscopic.

PLATE VII

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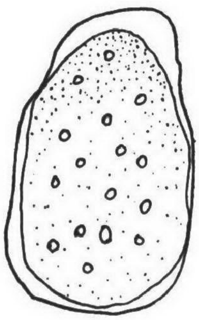
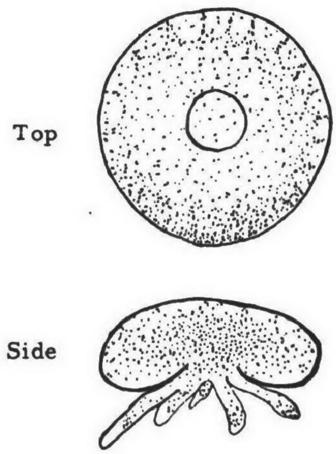
PLANKTONIC PROTOZOA

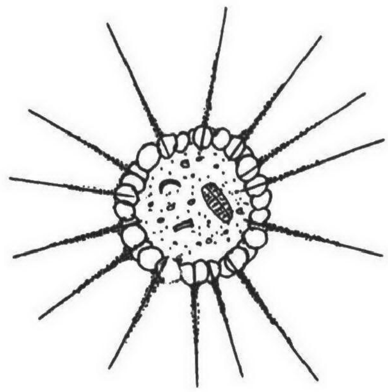
Peranema trichophorum



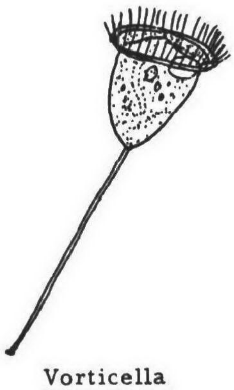
Chaos



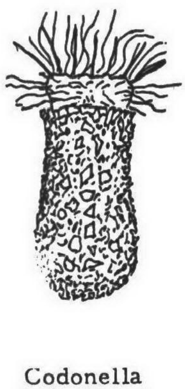
Arcella vulgaris



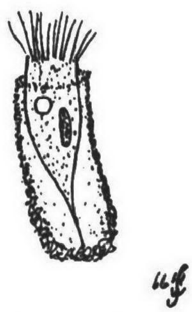
Actinosphaerium



Vorticella



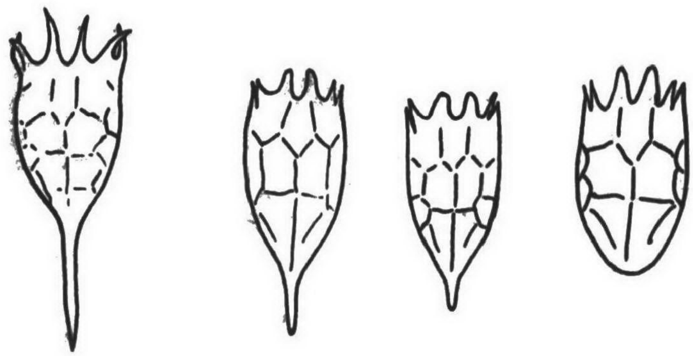
Codonella cratera



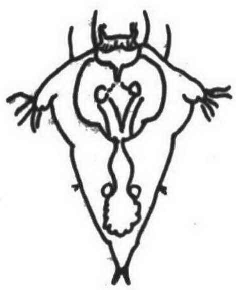
Tintinnidium fluviatle

PLATE VIII

PLANKTONIC ROTIFERS

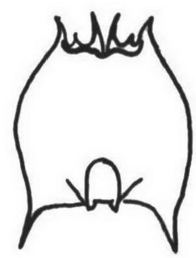


Various Forms of Keratella cochlearis



Synchaeta pectinata

Polygarthra vulgaris



Brachionus quadridentata

Rotaria sp

PLATE IX

FREE LIVING NEMATHELMINTHES, OR ROUND WORMS

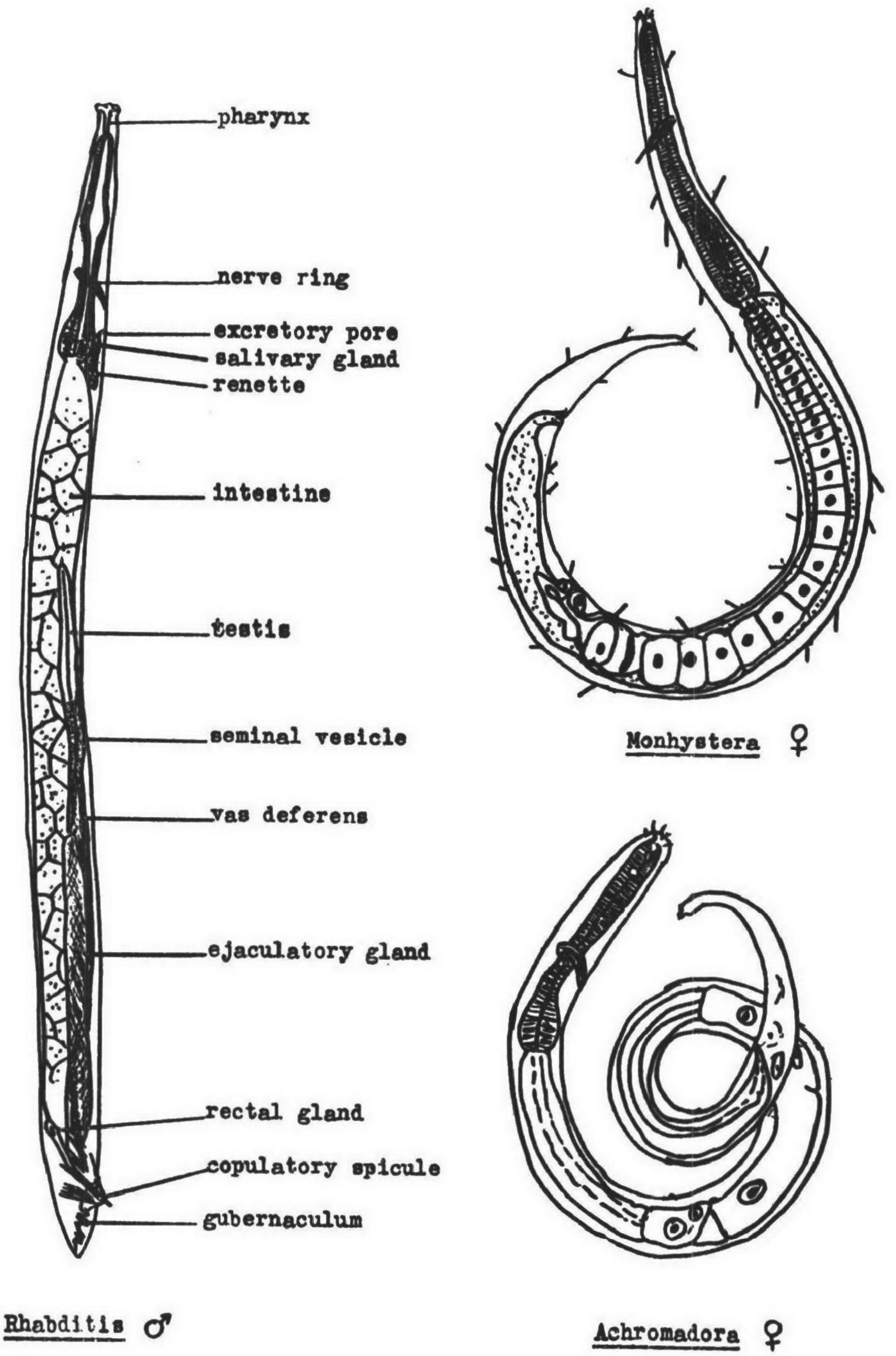
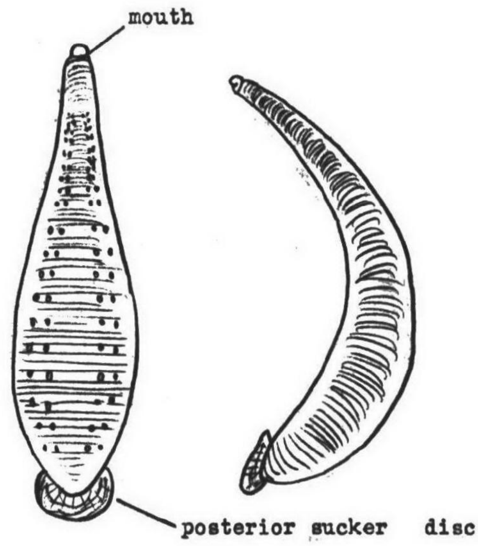


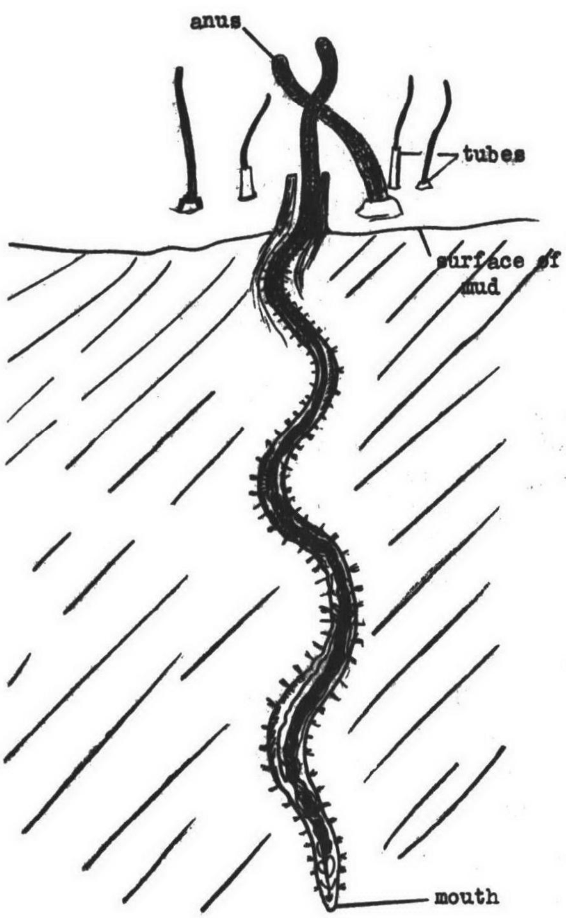
PLATE X

FRESH WATER ANNELID WORMS

Phylum Annelida



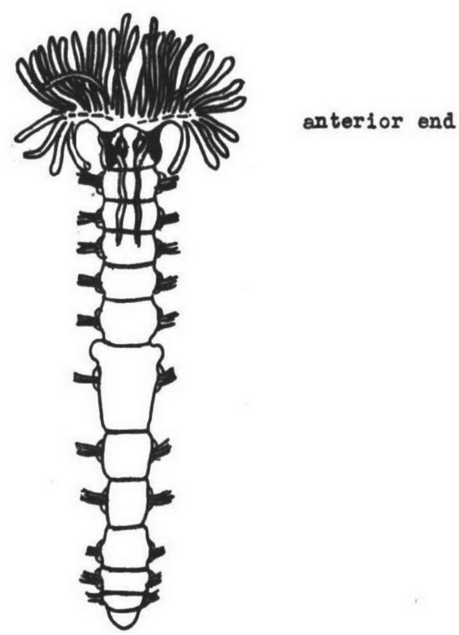
Class Hirudinea, leeches
(After Hegner)



Class Oligochaeta, earthworms

Ex: Tubifex , the sludgeworm

(After Liebman)



Class Polychaeta , polychaet worms

Ex: Manayunkia, a minute, rare, tube building worm.

(After Leidy)

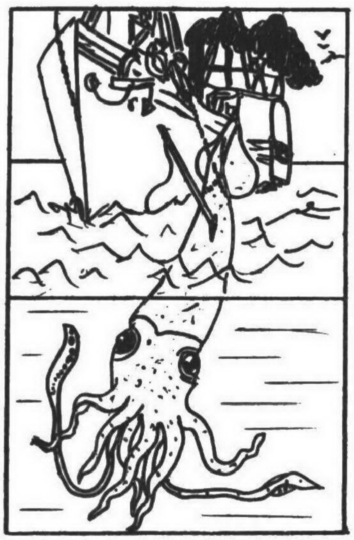
H.W.Jackson

PLATE XI

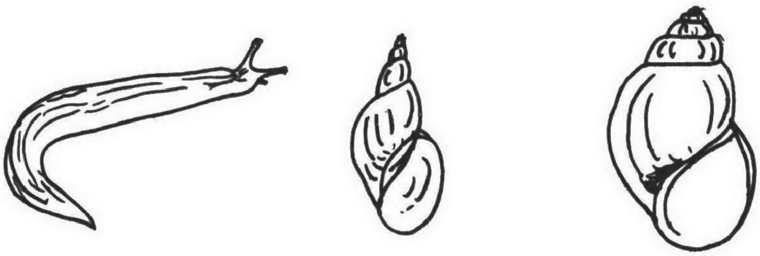
SOME MOLLUSCAN TYPES

Class: Cephalopoda.

Squids, octopus, cuttlefish.

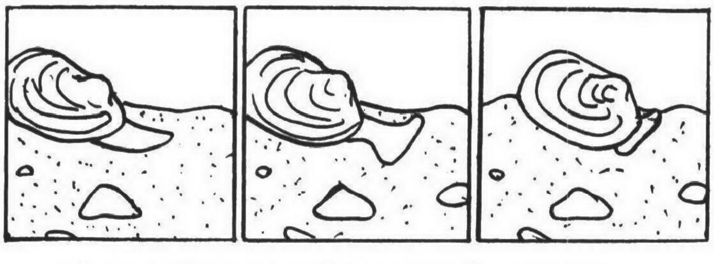


Exclusively marine. The giant squid shown was captured in the Atlantic in the early ninteenth century. (After Hegner)



Limax,	Lymnaea	Campeloma
a slug	an air breathing snail	a water breathing snail

Class: Gastropoda; snails and slugs. (After Buchsbaum)



Class: Pelecypoda; clams, mussels, oysters.

Locomotion of a freshwater clam, showing how foot is extended, the tip expanded, and the animal pulled along to its own anchor. (After Buchsbaum)

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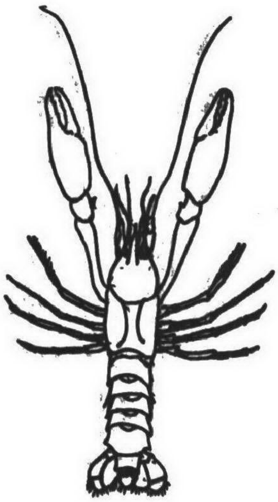
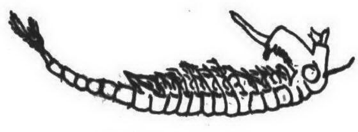
PLATE XII

Class CRUSTACEA

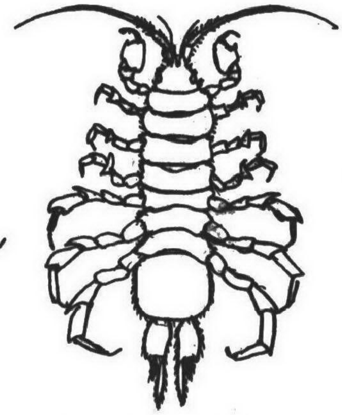
3/4



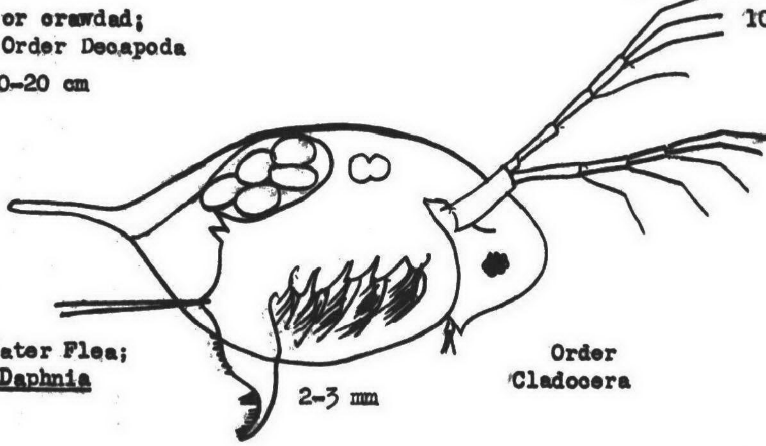
Crayfish, or crawdad;
Cambarus, Order Decapoda
10-20 cm



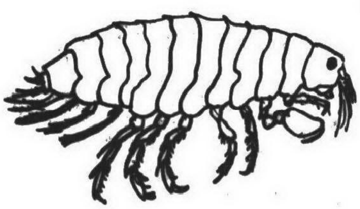
Fairy Shrimp;
Eubranchipus, Order
Phyllopoda
20-25 mm



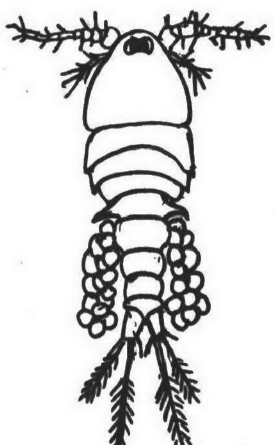
Sow Bug; Asellus,
Order Isopoda
10-20 mm



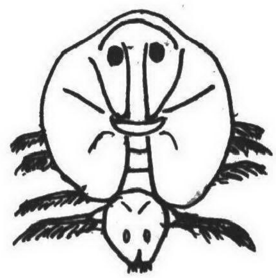
Water Flea;
Daphnia
2-3 mm
Order
Cladocera



Scud; Hyalella
Order Amphipoda
10-15 mm



Copepod; Cyclops, Order Copepoda
2-3 mm

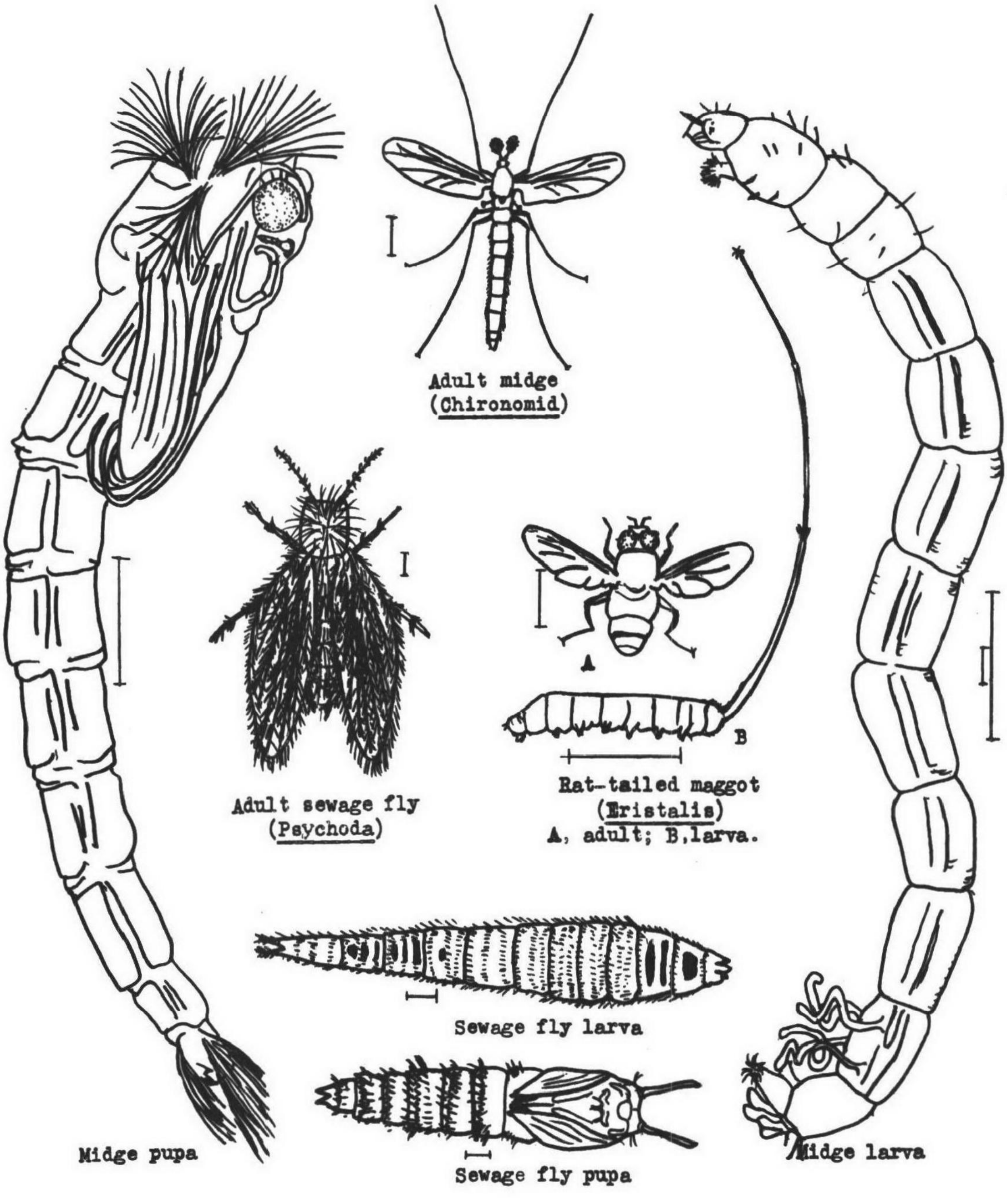


Fish Louse, Argulus;
a parasitic Copepod
5-6 mm

H.W.Jackson

PLATE XIII

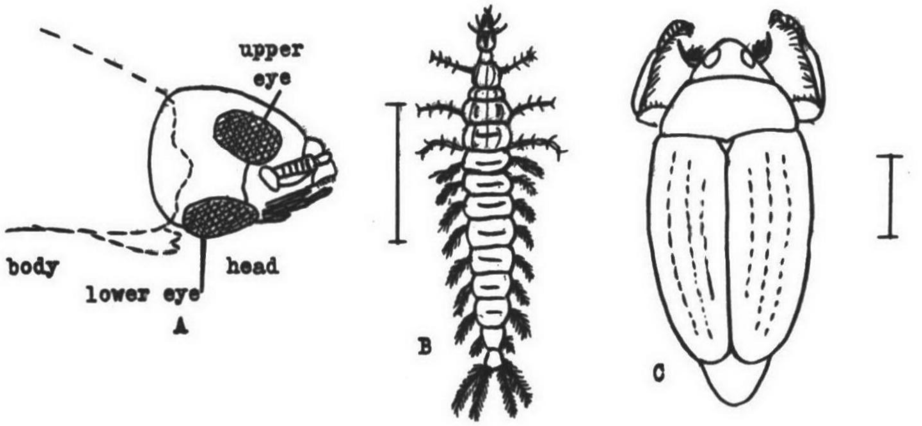
Two Winged Flies
Order DIPTERA



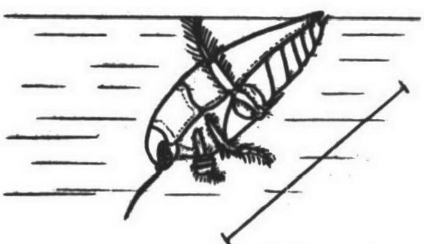
H.W.Jackson
After various authors

PLATE XIV

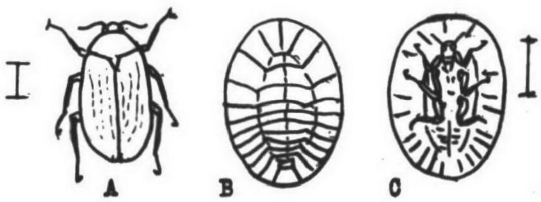
Beetles
Order COLEOPTERA



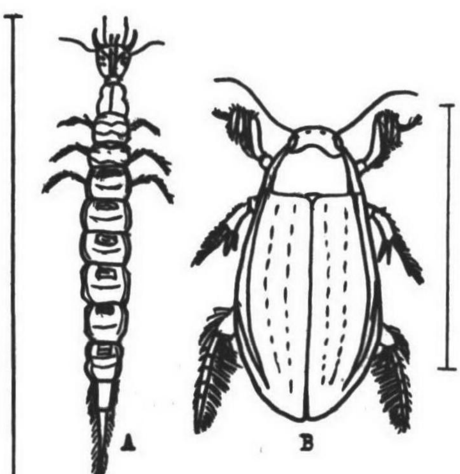
Whirligig beetle (Gyrinus) A, Side view of head of adult showing divided eye; B, Larva; C, Adult. Carnivorous.



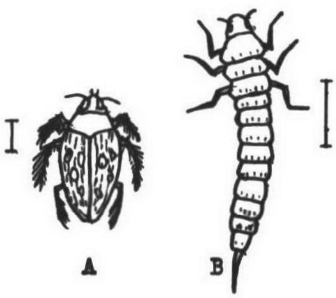
A diving beetle (Dytiscus) taking air at the surface.



The riffle beetle (Psephenus); A, adult; B, dorsal side of larva; C, ventral side of larva. Predominantly herbivorous.



A diving beetle (Cybister). The diving beetles include some of the largest and most voracious of aquatic insects. A, larva; B, adult.



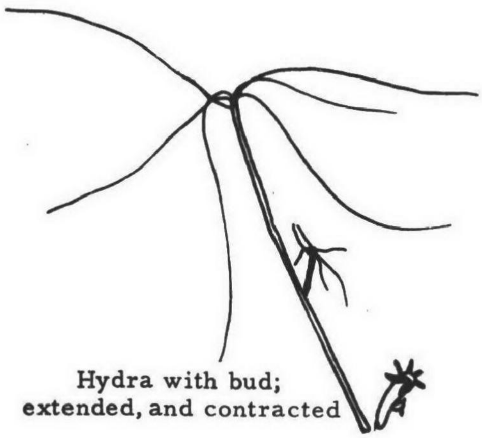
Crawling water beetle; A; adult; B, larva. Predominantly herbivorous.

H.W. Jackson. After Needham, Pennak, Morgan, and others.

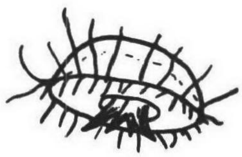
PLATE XV

MINOR PHYLA

Phylum Coelenterata

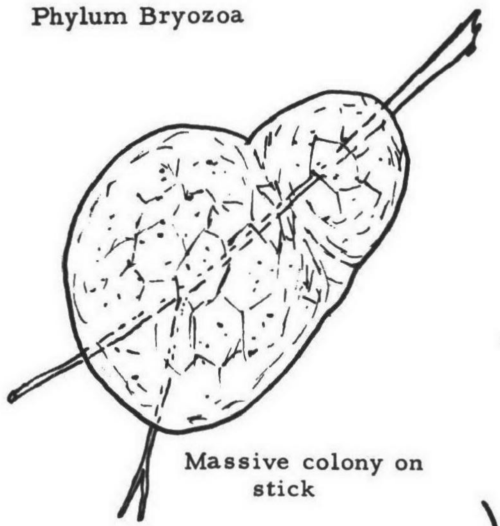


Hydra with bud; extended, and contracted

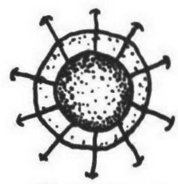


Medusa of <u>Craspedacusta</u>

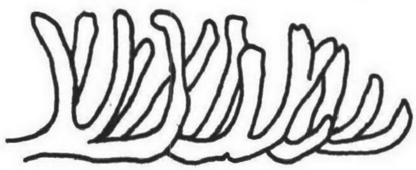
Phylum Bryozoa



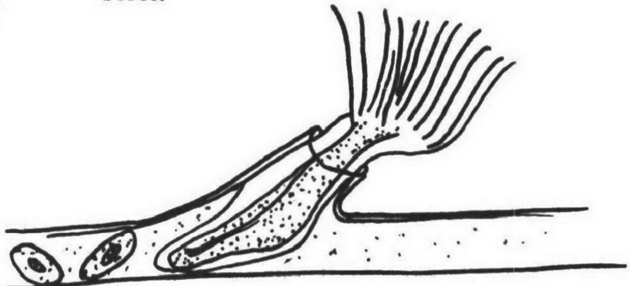
Massive colony on stick



Statoblast



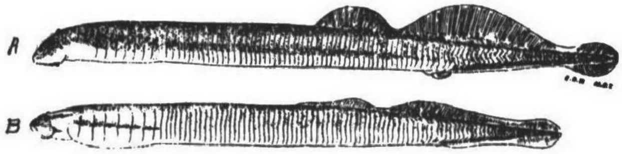
Creeping colony on rock



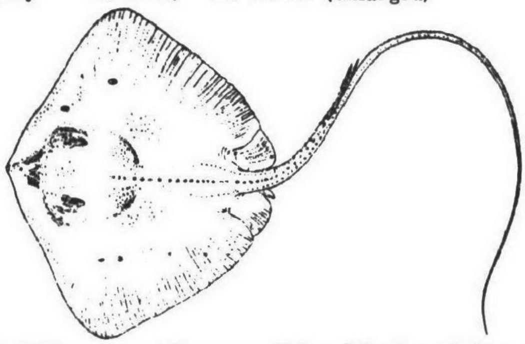
Single zooid, young statoblasts in tube

PLATE XVI

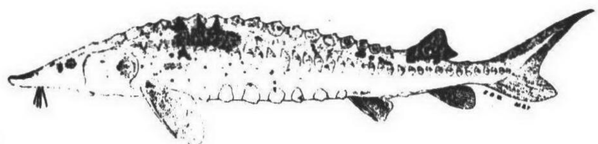
SOME PRIMITIVE FISHES



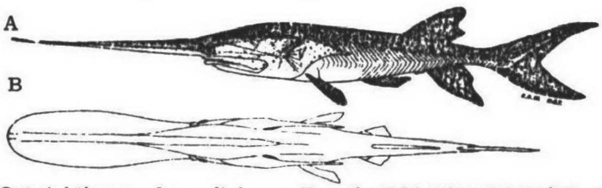
Class Agnatha, jawless fishes (lampreys and hagfishes) - Family PETROMYZONTIDAE, the lampreys. Lampetra aepyptera, the Brook Lamprey A: adult, B: larva (enlarged)



Class Chondrichthyes - cartilagenous fishes (sharks, skates, rays) Family DASYATIDAE - stingrays. Dasyatis centroura, the Roughtail Stingray

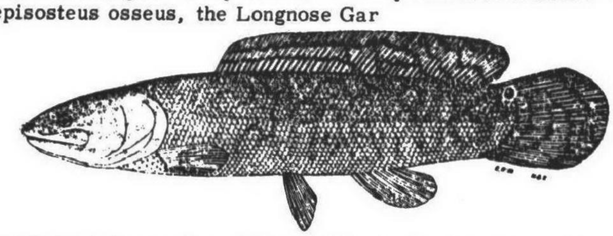


Class Osteichthyes - bony fishes - Family ACIPENSERIDAE, sturgeon. Acipenser fulvescens, the Lake Sturgeon



Class Osteichthyes - bony fishes - Family POLYODONTIDAE, the paddlefishes. Polyodon spathula, the Paddlefish. A:side view B:top view

Class Osteichthyes - bony fishes - Family LEPISOSTEIDAE - gars Lepisosteus osseus, the Longnose Gar



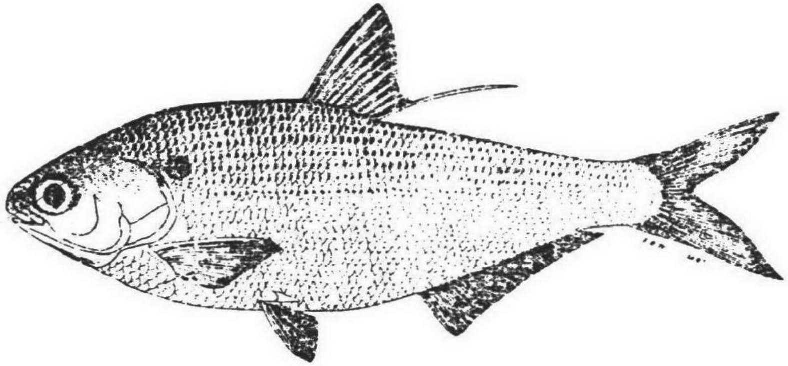
Class Osteichthyes - bony fishes - Family AMIIDAE, bowfins Aniia calva, the Bowfin

BI. AQ. pl. 91. 6. 60

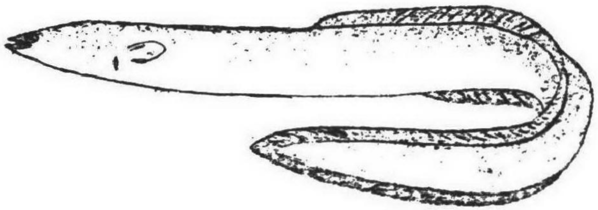
PLATE XVII

PLATE XVIII

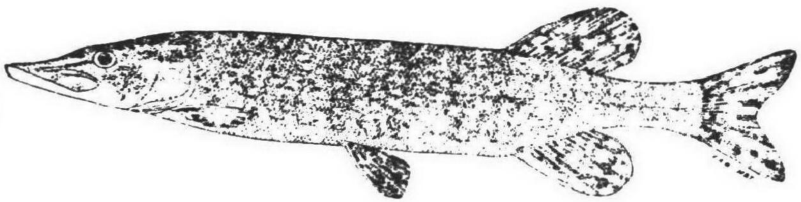
TYPES OF BONY FISHES



Family CLUPEIDAE - herrings
Dorosoma cepedianum - the eastern gizzard shad



Family ANGUILLEDAE - freshwater eels
Anguilla rostrata - the American eel



Family ESOCIDAE - pikes
Esox lucius - the northern pike

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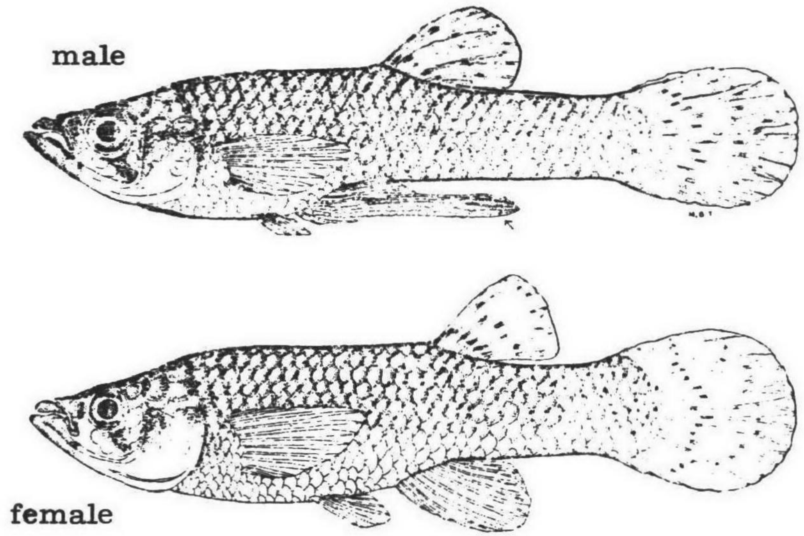
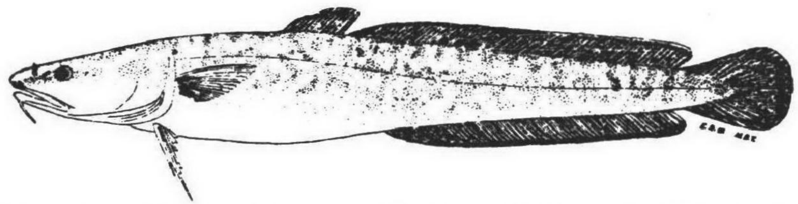
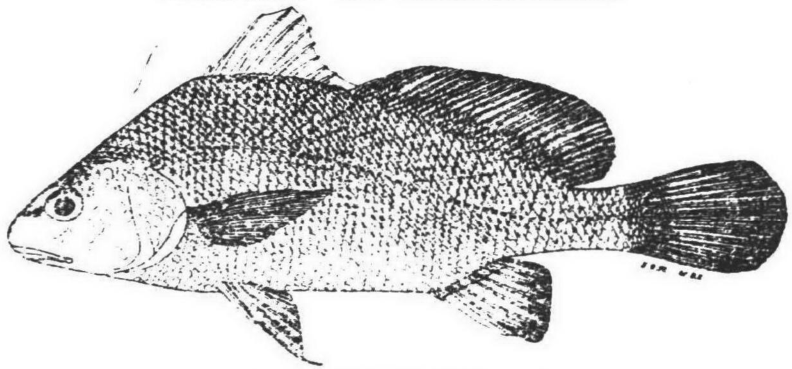


Family POECILIIDAE - livebearers
Gambusia affinis - the mosquitofish



Family GADDIDAE - codfishes, hakes, haddock, burbot
Lota lota - the eastern burbot



Family SCIAENIDAE - drums
Aplodinotus grunniens - the freshwater drum

SECTION B

PLANTS: PRODUCERS

The producers in both aquatic and terrestrial communities are the photosynthetic plants, autotrophic in function, which produce "fixed" energy and carbon in the forms of their own structure, and also oxygen that consumers can then use. Included in this section are surveys of the aquatic plant types from algae to vascular plants with keys for identification. All studies of productivity must take into account the four major plant life groups phytoplankton, benthic algae, periphyton, (in part) and macrophytes. Excessive or unwanted production of any one or combinations of these plant life groups is now a serious problem in environmental management.

Contents of Section B

TYPES OF ALGAE

I INTRODUCTION

A Algae in general may be defined as small pigmented plant-like organisms of relatively simple structure Actually the size range is extreme from only a few microns to over three hundred feet in length Commonly observed examples include the greenish pond scum or frog spittle of freshwater ponds, much of the golden brown slime covering rocks in a trout stream, and the great marine kelps and seaweeds Large freshwater forms as Nitella and Chara or stonewort are also included.

B Algae approach ubiquity in distribution. In addition to the commonly observed bodies of water, certain algae also live in such unlikely places as thermal springs, the surface of melting snow, on the hair of the three toed sloth in Central America, and in conjunction with certain fungi to form lichens

II ALGAE WILL BE GROUPED FOR THE SAKE OF CONVENIENCE INTO FOUR GENERAL TYPES

A Blue-greens (See plate Blue-Green Algae, Cyanophyceae) This is a valid technical group The size range is not very great, some being so small as to approach the size range of the bacteria

1 These are the only algae in which the pigments are not localized in definite bodies but dissolved throughout the cell Blue, red, or other pigments are present in addition to chlorophyll thus giving the cells a bluish green, yellow, or red color, at least enmasse

2 The nucleus lacks a nuclear membrane

3 Tend to achieve nuisance concentrations more frequently in the warm summer months and in the richer waters.

4 Vegetative reproduction, in addition to cell division, includes the formation of "hormogones," or short specifically delimited sections of trichomes (filaments)

5 Spores of three types are encountered

a Akinetes are usually larger, thick walled resting spores

b Heterocysts appear like empty cell walls, but are actually filled with protoplasm, have occasionally been observed to germinate

c Endospores, also called "gonidia" or conidia, are formed by repeated division of the protoplast within a given cell wall Present in only a few genera.

6 Some common examples of blue green algae are

Anacystis (Microcystis or Polycystis), Anabaena, Aphanizomenon, and Oscillatoria,

B The Pigmented flagellates (in contrast to the non-pigmented or animal-like flagellates) are a heterogeneous collection of motile forms from several different algal groups (See plate Flagellated algae)

1 There may be one, two, four, or more flagella per cell

2 There is a well organized nucleus

3 A light-sensitive red eyespot usually present

4 The chlorophyll is contained in one or more distinctive bodies called plastids.

5 Two or more cells may be associated in a colony

6 Non-motile life history stages may be encountered

7 Masses of stored starch called pyrenoid bodies are often conspicuous

8 Some examples of pigmented flagellates are· Euglena, Phacus, Chlamydomonas, Gonium, Volvox, Peridinium, Ceratium Mallomonas, Synura and Dinobryon

C The Non-motile green algae constitute another heterogeneous assembly of unrelated forms (See plate Non-Motile Green Algae)

1 Like the flagellates they have well organized nuclei and chloroplasts. The shape of the chloroplast is often distinctive

2 They lack flagella or any other locomotor device.

3 There is extreme structural variation among the group.

4 Some types tend to occur as a general planktonic mass or "bloom," often in combinations of two or more species

Some examples are Sphaerocystis, Pediastrum, Scenedesmus, and the desmid Cosmarium

5 Threadlike (filamentous) green algae may form masses or blankets, cutting off light, and reducing water circulation. They also add considerably to the total mass of organic matter Some examples of this type are Spirogyra, Hydrodictyon, Cladophora, Oedogonium, and Chara.

D The Diatoms constitute another valid technical group (See plate Diatoms-Bacillariophyceae)

1 In appearance, they are geometrically regular in shape The presence of a brownish pigment in addition to the chlorophyll gives them a golden to greenish color

2 Motile forms have a distinctive hesitating progression.

3 The most distinctive structural feature is the two-part shell (frustule) composed of silicon dioxide (glass).

a One part fits inside the other as the two halves of a pill box, or a petri dish.

b The surface of these shells are sculptured with minute pits and lines arranged with geometrical perfection

c The view from the side is called the "girdle view," that from above or below, the "valve view"

4 There are two general shapes of diatoms, circular (centric) and elongate (pennate) The elongate forms may be motile, the circular ones are not.

5 Diatoms may associate in colonies in various ways.

6 Examples of diatoms frequently encountered are Stephanodiscus Cyclotella, Asterionella, Fragilaria, Tabellaria, Synedra, and Nitzschia

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

KEY FOR IDENTIFICATION OF GROUPS OF FRESHWATER ALGAE

Beginning with "1a" and "1b", choose one of the two contrasting statements and follow this procedure with the "a" and "b" statements of the number given at the end of the chosen statement. Continue until the name of the algal group is given instead of another key number.

1a. Plastid (separate color body) absent, complete protoplast pigmented, generally blue-green, iodine starch test* negative ------------------------------ Blue-green algae

1b. Plastid or plastids present, parts of protoplast free of some or all pigments, generally green, brown, red, etc., but not blue-green, iodine starch test* positive or negative-------- 2

2a. Cell wall permanently rigid (never showing evidence of collapse), and with regular pattern of fine markings (striations, etc.), plastids brown to green, iodine starch test* negative, flagella absent, wall of two essentially similar halves, one placed over the other as a cover-----------------Diatoms

2b. Cell wall, if present, capable of sagging, wrinkling, bulging or rigidity, depending on existing turgor pressure of cell protoplast, regular pattern of fine markings on wall generally absent, plastids green, red, brown, etc., iodine starch test* positive or negative, flagella present or absent, cell wall continuous and generally not of two parts----------------- 3

3a. Cell or colony motile, flagella present (often not readily visible), anterior and posterior ends of cell different from one another in contents and often in shape-------------------Flagellate algae

3b. Non-motile, true flagella absent, ends of cells often not differentiated-------------------Green algae and associated forms

*Add one drop Lugol's (iodine) solution, diluted 1-1 with distilled water. In about 1 minute, if positive, starch is stained blue and, later black. Other structures (such as nucleus, plastids, cell wall) may also stain, but turn brown to yellow.

CMP

COMPARISON OF FOUR MAJOR GROUPS OF ALGAE

	Blue-Green	Pigmented flagellates	Greens	Diatoms
Color	Blue-Green (Brown)	Green Brown	Green	Brown (Light-Green)
Location of pigment	Throughout cell	In plastids	In plastids	In plastids
Starch	Absent	Present or Absent	Present	Absent
Slimy coating	Present	Absent in most	Absent in most	Absent in most
Nucleus	Absent	Present	Present	Present
Flagellum	Absent	Present	Absent	Absent
Cell Wall	Inseparable from slimy coating	Thin or Absent	Semi-rigid smooth or with spines	Very rigid, with regular markings
"Eye" spot	Absent	Present	Absent	Absent

BLUE-GREEN ALGAE

I WHAT ARE THE BLUE-GREEN ALGAE?

The blue-green algae (Myxophyceae) comprise that large group of microscopic organisms living in aquatic or moist habitats, carrying on photosynthesis and having differentiation of cells which is a little more complex than bacteria, and simpler than all of the other plants called algae

II WHY ARE THEY CALLED BLUE-GREEN:

In addition to the green photosynthetic pigment (chlorophyll-a) they always have a blue pigment (phyocyanin-c) which tends to give the cushions or mats they may form a blue-green tinge.

III WHERE ARE THE BLUE-GREENS FOUND?

Some are free floating (pelagic and planktonic), others grow from submerged or moist soil, rocks, wood and other objects in both freshwater and marine habitats.

IV WHAT ARE SOME OF THEIR GENERAL CHARACTERISTICS?

Some are gelatinous masses of various shapes floating in water. Others, microscopic in size, grow in great numbers so as to color the water in which they live. Structurally their cells are similar to bacteria. Their protoplasts may be sheathed or imbedded in gelatin, making them slimy Cells of blue-green algae are without organized nuclei, central vacuoles, or cilia and flagella. No sexual reproduction is known. Asexual reproduction may be effected by fragmentation, in which case special separation devices are formed (dead cells, and heterocysts). Some species are preserved over unfavorable periods by special spores (akinetes and endospores).

V OF WHAT IMPORTANCE ARE BLUE-GREEN ALGAE?

They have both positive and negative economic significance Because they can convert radient energy into chemical energy, they are producers forming a first link at the base of the food chain. Because many very intricate nutritional relationships exist among the myraids of organisms it is difficult to know the value of the blue-greens However, people who know what the blue-greens can do to drinking and recreational water classify them as of negative economic importance, because they are often nuisances when they impart color, bad odors, and fishy tastes, or toxins. Some of them can foul pipes and clog filters.

VI WHEN ARE THEY MOST COMMON?

They are widely distributed in time and space, but tend to reach nuisance concentrations more frequently in the late summer and in eutrophic waters.

VII WHAT DO BLUE-GREEN ALGAE DO FOR A LIVING?

The pioneer-forms are of great ecological importance because they live in habitats frequented by few other forms of live, synthesizing organic substances and building substrata that can support other kinds of life.

A Some blue-greens live in association with other organisms as symbionts. Still others are found in polluted waters because they are able to exist in habitats poor in oxygen. The growth of these kinds of algae under such conditions tends to make a polluted condition worse

B On the other hand some species should be promoted because they provide oxygen and food through photosynthesis. The first evident product of photosynthesis is glycogen, and is the cause of the brown coloration with the iodine test. Some of the glycogen is used to produce glycoproteins. The gelatinous sheath is composed of pectic substances, cellulose and related compounds

C When blue-greens mat at the surface of the water the increased lighting may be too strong, resulting in a kill. At this time they may turn from a blue-green to a yellow-green color. Here they decompose in mass. The resulting intermediate products of decomposition may be highly undesirable, because of bad looks, four odors, bad tastes and toxins. Under these conditions the BOD may produce conditions not unlike raw sewage.

VIII WHAT DO BLUE-GREEN ALGAE LOOK LIKE UNDER THE MICROSCOPE?

A A cross section of a typical cell would show an outside nonliving gelatinous layer surrounding a woody cell wall, which is bulging from turgor pressure from the cell (plasma) membrane, pushing the wall outwardly. The protoplasm, contained within the plasma membrane, is divided into two regions The peripheral pigmented portion called chromatoplasm, and an inner centroplasm, the centroplasm contains chromatins, which is also known as in incipient nucleus or central body, containing chromosomes and genes. Structures (chromatophores or plastids) containing pigments have not been found in the blue-greens The photosynthetic pigments are dissolved in the peripheral cytoplasm, which is known as the chromatoplasm.

B A simple way to understand the cross section would be to compare it with a doughnut, with the hole representing the colorless central body or incipient nucleus, which houses the chromatoplasm, having the characteristic blue-green color from its dissolved photosynthetic pigments

IX WHAT CAUSES THESE FOUL-TO-SMELL UNSIGHTLY BLOOMS?

When the protoplasts become sick or old they may develop a great number of "pseudovacuoles" filled with gas. These gas bubbles make the algae buoyant in such a way that they may "flower" or bloom by rising to the surface (planktonic, healthy blue-greens normally possess pseudovacuoles, which are here excepted). Soon they begin to stink because of the odors produced from putrefaction. The lack of dissolved oxygen during this period may **affect** other organisms.

X ARE ALL BLOOMS PUTREFACTIVE?

No. Healthy blooms are produced by myraids of cells living near the surface of the water at times when environmental conditions are especially favorable for them. Putrefactive blooms are usually from masses of algae undergoing degradation.

XI WHAT ARE SOME OF THE MAJOR KINDS OF BLUE-GREENS?

Most species of blue-greens may be placed into two major groups the nonfilamentous (coccoid) forms, and the filamentous forms. See the set of drawings following this treatment to get a graphic concept of the two groups.

XII WHAT ARE SOME OF THE MORE DISTINCTIVE FEATURES OF BLUE-GREENS?

A In comparing the blue-greens with other algae it is easier to tell what they do not possess than what they do. They do not have chromatophores or plastids, cilia, flagella, organized nuclei, gametes, central vacuoles, chlorophyll-b, or true starch.

B Many of the filamentous forms, especially the Oscillatoriaceae, exhibit an unexplained movement. When the filamentous forms are surrounded by a gelatinous sheath the row of cells inside is called a <u>trichome</u>, and the trichome with its enclosing sheath is called a filament. There may be more than one trichome within a sheath.

True branching occurs when a cell of the series divides lengthwise and the outer-formed cell adds cells to form a true branch. However, two or more trichomes within a single sheath may be so arranged that though they appear to be branches, their cells actually have all divided in the same plane, and the trichomes have pushed out from growth to form false branching, as in Tolypothrix.

C An occasional reticulated or bubbly appearance is referred to as pseudovacuolation, and en mass imparts a pale, yellowish color to the algae. Under low powers these vacuoles appear dark, under higher magnifications they are reddish.

D Vegetative reproduction in addition to cell division for the unicellular forms, is by special kinds of fragmentation. This includes the formation of hormogones, which are specifically delimited sections of trichomes, and are characteristic of some taxonomic entities.

E *Spores of three types are encountered.*

1 Akinetes are usually larger, non-motile, thick-walled resting spores.

2 Heterocysts appear like empty cell walls, but are filled with colorless protoplasm and have been occasionally observed to germinate.

3 Endospores, also called gonidia, are formed by a repeated division of the protoplast within a cell wall container.

XIII WHAT ARE SOME EXAMPLES OF BLUE-GREEN ALGAE?

A Anacystis (Microcystis) is common in hard waters.

1 Colonies are always free floating.

2 Their shapes may be roughly spherical or irregular, microscopic or macroscopic.

3 The gelatinous matrix may be extremely transparent, easily broken up on preservation.

4 They frequently contain pseudovacuoles.

B Anabaena is an example of a filamentous form.

1 Filaments may occur singly or in irregular colonies, and free floating or in a delicate nucous matrix.

2 Trichomes have practically the same diameter throughout, may be straight, spiral, or irregularly contorted

3 Cells are usually spherical, or barrel shaped, rarely cylindrical and never discoid.

4 Heterocysts are usually the same shape but are slightly larger than the vegetative cells.

5 Akinetes are always larger than the vegetative cells, roughly cylindrical, and with rounded ends.

6 It may be readily distinguished from Nostoc by the lack of a firm gelatinous envelope.

7 It may produce an undesirable grassy, moldy or other odor.

C Aphanizomenon is a strictly planktonic filamentous form.

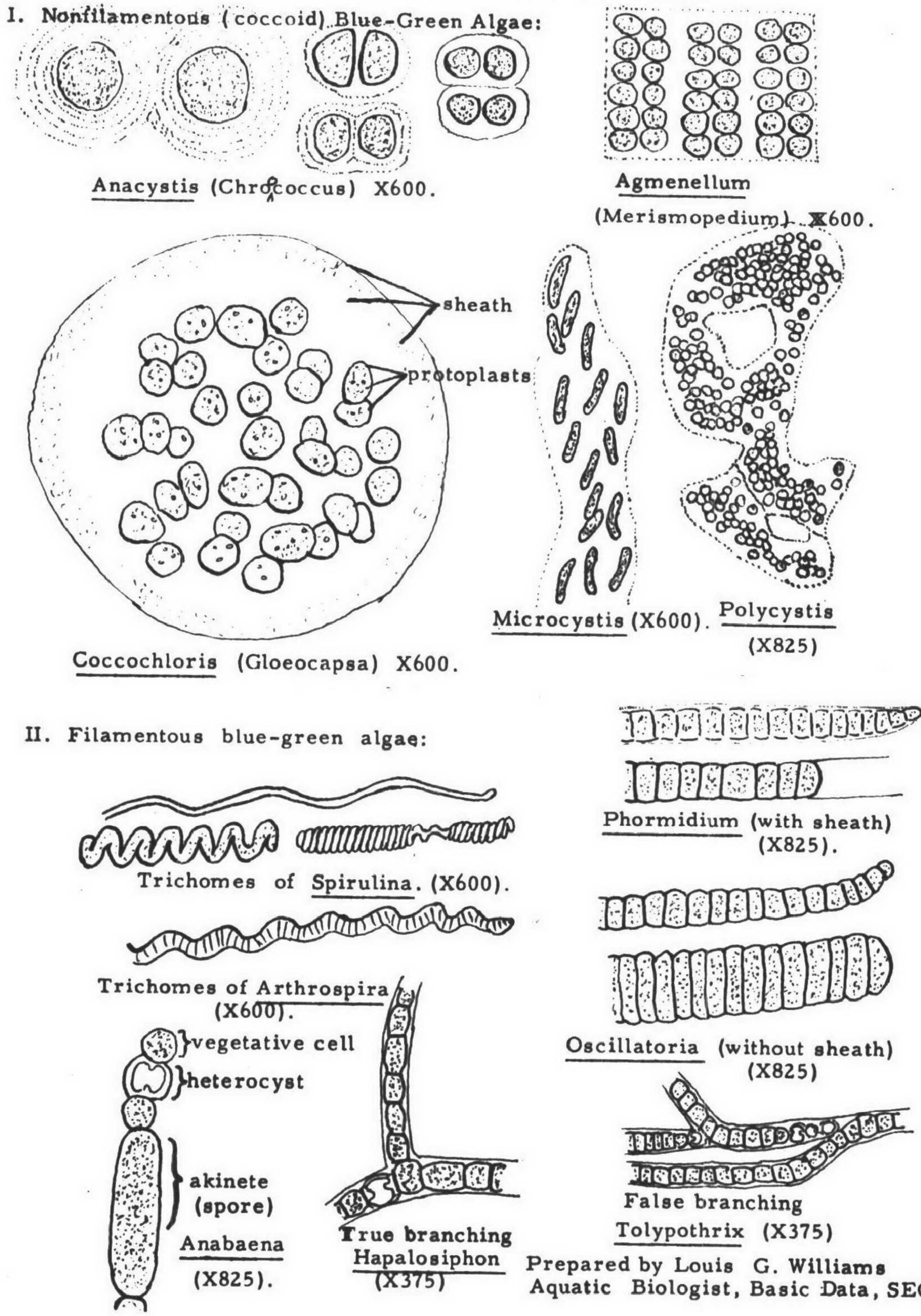
1 Trichomes are relatively straight, and laterally joined into loose macroscopic free-floating flake-like colonies

2 Cells are cylindrical or barrel shaped, longer than broad.

3 Heterocysts occur within the filament (i.e., not terminal).

4 Akinetes are cylindrical and relatively long.

SOME BLUE-GREEN ALGAE



Prepared by Louis G. Williams
Aquatic Biologist, Basic Data, SEC.

5 Often imparts grassy or nasturtium-like odors to water.

D Oscillatoria is a large and ubiquitous genus.

1 Filaments may occur singly or interwoven to form mats of indefinite extent.

2 Trichomes are unbranched, cylindrical, and practically without sheaths.

3 Species with narrow trichomes have long cylindrical cells while those with broader trichomes have short broad cells.

4 No heterocysts or akinetes are known in Oscillatoria. It reproduces by fragmentation from hormongonia only.

5 Live species exhibit "oscillatoria" movements, which are oscillating.

6 Species of Oscillatoria may be readily distinguished from Lyngbya by the absence of a sheath.

E Nodularia is an occasional producer of blooms.

1 Vegetative cells, heterocysts, and even the akinetes are broader than long.

2 Trichomes are practically the same diameter throughout.

3 Sheaths are usually distinct, fairly firm, and with a single trichome.

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GREEN AND OTHER PIGMENTED FLAGELLATES

I INTRODUCTION

A A flagellate is a free swimming cell (or colony) with one or more flagella.

B Motile flagellated cells occur in most (not all) great groups of plants and animals.

C Out main concern will be with "mature" flagellated algae.

II THE STRUCTURE OF A PIGMENTED OR PLANT-LIKE FLAGELLATE

A There is a well organized nucleus.

B The flagellum is a long whip-like process which acts as a propeller.

1 It has a distinctive structure.

2 There may be one or several per cell.

C The chlorophyll is contained in one or more chloroplasts.

D Two or more cells may be associated in a colony.

E Non-Motile Life history stages may be encountered.

F Size is of little use in identification.

G Pyrenoid bodies are often conspicuous.

III The Euglenophyta or Euglena-like algae (Figures 1-4) are almost exclusively single celled free swimming flagellates. Nutrition may be holophytic, holozoic, or saprophytic, even within the same species. Referred to by zoologists as mastigophora, many animal like forms are parasitic or commensalistic. Food reserves of plant-like forms are as paramylin (an insoluble carbohydrate) and fats (do not respond to starch test). Thick walled resting stages (cysts) are common. "Metabolic movement" characteristics of some genera (Euglena).

Eyespot usually present in anterior end, rarely more than one flagellum.

A Euglena is a large genus with pronounced metabolic movement (Figure 1).

1 Cells spindle shaped

2 Single flagellum

3 Eyespot usually present

4 Chloroplasts numerous, discoid to band shaped

5 E. sanguinea has red pigment.

6 E. viridis generally favors water rich in organic matter.

7 E. gracilis is less tolerant of pollution.

B Phacus cells maintain a rigid shape (Figure 2).

1 Often flattened and twisted, with pointed tip or tail end.

2 Cell wall (periplast) often marked with fine ridges.

3 P. pyrum favored by polluted water.

4 P. pleuronectes relatively intolerant of pollution.

C Trachelomonas cells surrounded by a distinct shell (lorica) with flagellum sticking through hole or collar (Figure 4).

1 Surface may be smooth or rough

2 Usually brown in color

3 Some species such as T. cerebea known to clog filters

D Lepocinclis has rigid naked cells with longitudinal or spiral ridges (Figure 3).

1 Cells uncompressed, elipsoidal to oval (in contrast to phacus)

2 Only two species with pointed tails

3 L. texta often associated with waters of high organic content

IV The Chlorophyta or grass green algae (Figures 5-9) are the largest and most varied group. Non-flagellated forms predominate but many conspicuous flagellates are included. Food reserves are usually stored as starch which is readily identified with iodine. Usually two flagella of equal length are present. More planktonic forms are included than in any other group, predominating in the late spring and early autumn.

The cell is typically surrounded by a definite wall and usually has a definite shape. Cell pigments closely resemble those of higher plants, but some have accessory pigments and a few forms have little or none. The chloroplasts always have a shape characteristic of the genus.

The flagellated chlorophyta are contained in the Order Volvocales, the Volcocine algae. All are actively motile during vegetative phases. May be unicellular or colonial. All have an eyespot near the base of the flagella. Colonies may range from a simple plate (Gonium sociale) to a complete hollow sphere (Volvox spp).

A Chlamydomonas is a solitary free swimming genus (Figure 5).

1 Species range from cylindrical to pearshaped.

2 Some species have a gelatinous sheath.

3 There are two flagella inserted close together.

4 Generally favored by polluted waters.

B Carteria resembles Chlamydomonas very closely except that it has four flagella instead of two. Generally favored by polluted water (Figure 7).

C Phacotus usually has free swimming biflagellate cells surrounded by biconcave envelopes resembling two clam shells. These are usually sculptured, dark colored, and impregnated with calcium carbonate.

1 The eyespot ranges from anterior to posterior.

2 Several daughter cells may be retained within the old envelopes of the parent cell.

3 A clean water indicator.

D Chlorogonium is a distinctive genus in which the cell is fusiform, the tail end pointed, and the anterior end slightly blunt (Figure 6).

1 The two flagella only about half as long as the cell.

2 The cell wall is rather delicate.

3 An eyespot usually present near the anterior end.

4 Favored by pollution.

E Gonium colonies typically have 4 to 32 cells arranged in a plate (Figure 8).

1 The cells are imbedded in a gelatinous matrix.

2 Sixteen celled colonies move through the water with a somersault-like motion.

3 Four and eight celled colonies swim flagella end first.

4 Gonium pectorale is typically a plankton form.

F Pandorina colonies range up to 32 cells, usually roughly spherical (Figure 9).

1 Cells arranged in a hollow sphere within a gelatinous matrix.

2 Often encountered especially in hard-water lakes, but seldom abundant.

3 P. morum may cause a faintly fishy odor.

G Eudorina has up to 64 cells in roughly spherical colonies.

1 The cells may be deeply imbedded in a gelatinous matrix.

2 Common in the plankton of soft water lakes.

3 E. elegans is widely distributed.

4 May cause faintly fishy odor.

H Pleodorina has up to 128 cells located near the surface of the gelatinous matrix. It is widespread in the United States.

I Volvox rarely has less than 500 cells per colony.

1 Central portion of the mature colony may contain only water.

2 Daughter colonies form inside the parent colony.

3 V. aureus imparts a fishy odor to the water when present in abundance.

J Chlamydobotrys has "mulberry shaped" colonies, with biflagellate cells alternately arranged in tiers of four each. (Spondylomorum has quadriflagellate cells).

1 There is no enveloping sheath.

2 C. stellata is favored by pollution.

V The Pyrrhophyta includes principally the armored or dinoflagellates (Dinophyceae) (Figures 14-16). This group is almost exclusively flagellated and is characterized by chromatophores which are yellow-brown in color. Food reserves are stored as starch or oil. Naked, holozoic, and saprozoic representatives are found. Both "unarmored", and "armored" forms with chromatophores are found to ingest solid food readily, and holozoic nutrition may be as important as holophytic.

The great majority have walls of cellulose consisting of a definite number of articulated plates which may be very elaborate in structure. There is always a groove girdling the cell in which one flagellum operates, the other extends backward from the point of origin.

Most of the dino-flagellates are marine and some are parasitic. There are six fresh water genera of importance in this country.

A Gymnodinum species are generally naked except for a few freshwater species.

G. brevis (marine) is a toxic form considered to be responsible for the "red tide" episodes in Florida and elsewhere.

B Species of Gonyaulax (catanella and tamarensis) are responsible for the paralytic shellfish poisoning.

C Ceratium is distinctive in that the anterior and posterior ends are continued as long horns (Figure 16).

1 Seasonal temperature changes have a pronounced effect on the shape of the cells of this species.

2 C. hirudinella in high concentration is reported to produce a "vile stench".

D Peridinium is a circular, oval, or angular form, depending on the view (Figure 15).

1 Cell wall is thick and heavy.

2 Plates are usually much ornamented.

3 P. cinctum has been charged with a fishy odor.

VI The Division Chrysophyta contains two classes which include flagellates, the Xanthophyceae or Heterokontae (yellow-green algae) and the Chrysophyceae (golden-green algae) (Figures 10-13). The third class, the diatoms (Bacillarieae or Bacillariophyceae), is not flagellated.

A None of the Xanthophyceae are included in the present discussion.

B The Chrysophyceae possess chromatophores of a golden brown color, usually without pyrenoids. Food reserves are stored as fats and leucosin. One or two flagella, if two, they may be of equal or unequal length. Internal silicious cysts may be formed. Tend to occur in relatively pure water. Both holozoic and holophytic types of nutrition are found. Certain minute forms considered to be highly sensitive to pollution.

1 Mallomonas is a solitary, free swimming genus with one flagellum (Figure 13).

a Covered with silicious plates, many of which bear long silicious spines.

b Tends to inhabit clear water lakes at moderate depths.

c M. caudata imparts a fishy odor to the water.

2 Chrysococcus cells are minute, with two yellowish brown chromatophores and one flagellum.

a Droplets of stored oil present

b Lorica distinct

c C. rufescens a clean water form

3 Chromulina has a single flagellum, may accumulate single large granule of leucosin at posterior end of cell (Figure 10).

C. rosanoffii is a clean water indicator.

4 Synura is a biflagellate form growing in radially arranged, naked colonies (Figure 11).

a Flagella equal in length

b Cells pyriform or egg shaped

c S. uvella produces a cucumber or muskmelon odor

5 Uroglenopsis forms free swimming colonies of approximately spherical biflagellate cells embedded near the periphery of a roughly spherical gelatinous matrix.

a Flagella are unequal in length.

b U. americana may range up to .5 mm in diameter, and contain 1000 or more cells.

c U. am. also causes strong fishy odor.

6 Dinobryon may be solitary or colonial, free floating or attached. Colonies are arborescent (Figure 12).

a Cells attached to bottom of open roughly cylindrical lorica or sheath.

b Two flagella of unequal length.

c Conspicuous eyespot usually present.

d Taxonomy of the group is involved.

e D. sertularia may clog filters.

f D. divergens may cause a fishy odor.

(fig 1 - 13 from Lackey and Callaway)

1

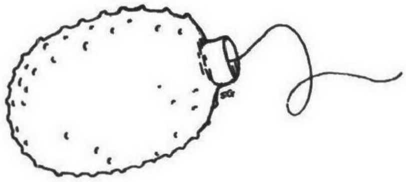
Euglena

2

Phacus

3

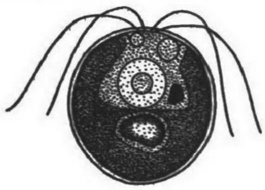
Lepocinclis

4

Trachelomonas

GREEN EUGLENOIDS

5

Chlamydomonas

7

Carteria

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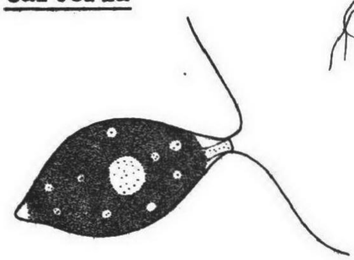
Chlorogonium

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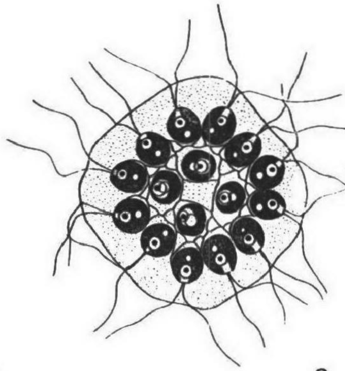
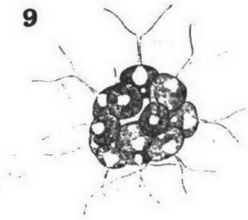
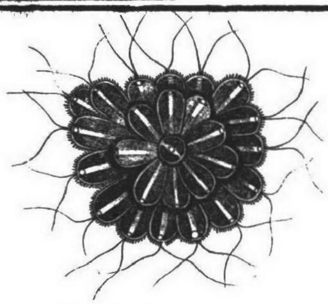

Gonium

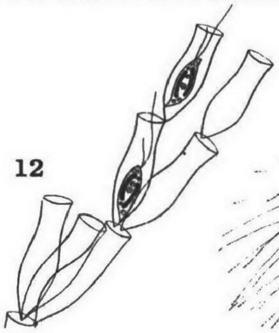
9

Pandorina

GREEN PHYTOMONADS

10

Chromulina

11

Synura

12

Dinobryon

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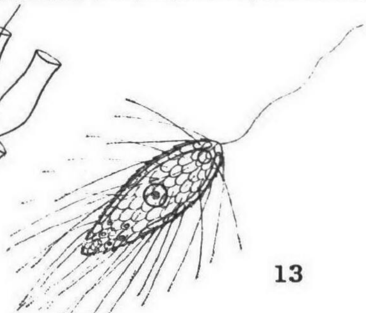
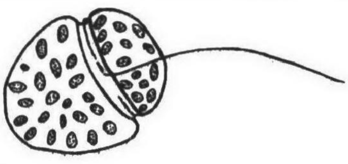
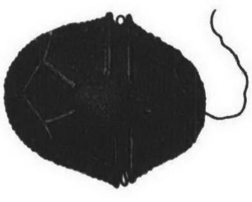
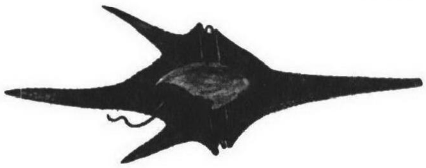

Mallomonas

YELLOW CHRYSOMONADS

14

Massartia

15

Peridinium

16

Ceratium

ams 3-70

YELLOW-BROWN DINOFLAGELLATES

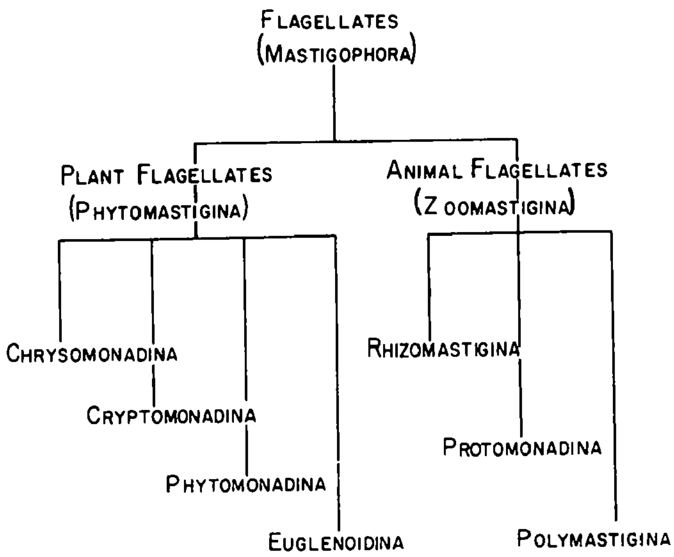


Figure 17 Phylogenetic Family Tree of the Flagellates

(from Calaway and Lackey)

VII There are two distinctive groups whose systematic position is uncertain, the chloromonads and the cryptomonads. Only one genus of the latter group is included here.

A Rhodomonas may range from bright red through pale brown to olive green.

1 Cells compressed, narrow at the posterior end

2 Two flagella of unequal length

3 R. lacustris a small form intolerant of pollution

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FILAMENTOUS GREEN ALGAE[1]

I MANY OF THESE FORMS ARE VISIBLE TO THE UNAIDED EYE

A They may be several inches or even a foot or more in length. In many cases they are not found as isolated filaments but develop in large aggregations to form floating or attached mats or tufts. The attached forms are generally capable of remaining alive after being broken away from the substrate.

B Included in the group are some of the most common and most conspicuous algae in freshwater habitats. A few of them have been given common names such as pond silk, green felt, frog-spawn algae, and stoneworts.

II CHARACTERISTICS OF FILAMENTOUS ALGAE

A These algae are in the form of cylindrical cells held together as a thread ("filament"), which may be in large clusters or growing separately. Some are attached to rocks or other materials while others are free. They may be unbranched ("simple") or branched, the tips are gradually narrowed ("attenuated") to a point. Some are surrounded by a mucilaginous envelope.

B Each cell is a short or long cylinder with a distinct wall. The protoplast contains a nucleus which is generally inconspicuous.

1 The plastid or chloroplast is the prominent structure. It contains chlorophyll and starch centers ("pyrenoids"), and varies in size, shape, and number per cell. It may be pressed against the wall ("parietal") or extend through the central axis of the cell ("axial").

2 Clear areas of cell sap ("vacuoles") are generally present in the cell.

C Specialized structures are present in some filaments.

1 Some filaments break up into "H" sections.

2 Apical caps are present in others.

3 Replicate end walls are present in some.

4 Some filaments are overgrown with a cortex.

5 Attached filaments have the basal cell developed into a "hold fast cell" (hapteron).

III REPRODUCTION MAY TAKE PLACE BY SEVERAL METHODS

A Cell division may occur in all cells or in certain selected ones.

B Spores called akinetes may be formed.

C Zoospores (motile) and aplanospores (non-motile) are common.

D Fragmentation of filaments may occur.

E Many kinds reproduce sexually, often with specialized gamete forming cells.

IV EXAMPLES OF FILAMENTOUS GREEN ALGAE ARE:

A Unbranched forms

*Spirogyra
*Mougeotia
Zygnema
Ulothrix
Microspora
Tribonema
Desmidium
Oedogonium

*Planktonic or occasionally planktonic

1 Including a few yellow-brown and red algae.

B Branched forms

Cladophora
Pithopora
Stigeoclonium
Chaetophora
Draparnaldia
Rhizoclonium
Audouinella
Bulbochaete
Nitella

C Specialized and related forms

Schizomeris
Comsopogon
Batrachospermum
Chara
Lemanea
Vaucheria

V Habitats include the planktonic growths as well as surface mats or blankets and benthic attached forms on rocks in riffles of streams, at the shoreline of lakes and reservoirs, concrete walls, etc.

A Attached forms may break loose to become mixed with plankton or to form floating mats.

B Cladophora mats are a nuisance on many beaches on the Great Lakes.

VI IMPORTANCE OF FILAMENTOUS GREEN ALGAE

A They may cause clogging of sand filters, intake screens, and canals.

B They may produce tastes and odors in water or putrid odor (also producing H_2S which damage painted surfaces) when washed ashore around lakes and reservoirs.

C They may cause unsightly growths or interfere with fishing and swimming in recreation areas.

D Some are useful as indicators of water quality in relation to pollution.

E Together with other algae, they release oxygen required by fish, and for self-purification of streams.

F They may produce a slime which interferes with some industrial uses of water such as in paper manufacture and in cooling towers.

VII CLASSIFICATION

A Ulotrichaceae

Ulothrix, Microspora, Hormidium

B Cladophoraceae

Cladophora, Pithophora, Rhizoclonium

C Chaetophoraceae

Chaetophora, Stigeoclonium, Draparnaldia

D Oedogeniaceae

Oedogonium, Bulbochaete

E Schizomeridaceae

1 Schizomeris

F Ulvaceae

Enteromorpha, Monostroma

G Zygnemataceae

Zygnema, Spirogyra, Mougeotia

H Desmidiaceae

Desmidium, Hyalotheca

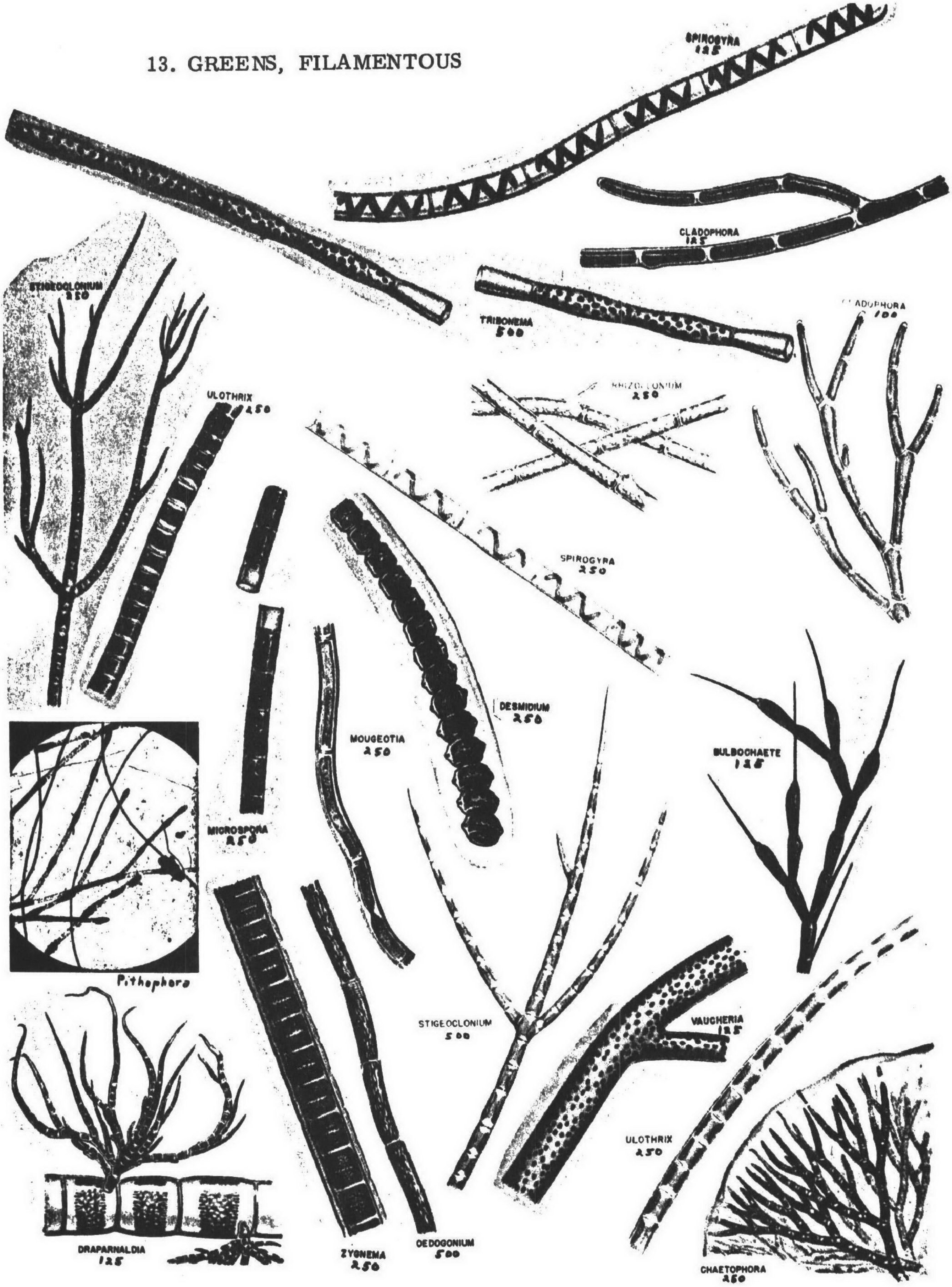
I Tribonemataceae

Tribonema, Bumilleria

J Characeae

Chara, Nitella, Tolypella

13. GREENS, FILAMENTOUS



VIII IDENTIFICATION

A Branching and attenuation are of primary importance.

B Plastids: shape, location and number per cell are essential.

C Other characteristics include grouping of filaments, gelatinous envelope and special features such as "H" shaped fragments.

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COCCOID GREEN ALGAE

I INTRODUCTION

For the sake of convenience, the non-motile green algae are to be discussed in two sections: those that tend to live as relatively discrete or free floating planktonic units, and those that tend to grow in masses or mats of material, often filamentous in nature, attached or free floating.

II The green or "grass green" algae is one of the most varied and conspicuous groups with which we have to deal. The forms mentioned below have been artificially grouped for convenience according to cell shape. Botanists would list these genera in several different categories in the family "Chlorophyceae."

These algae typically have a relatively high chlorophyll content, and the food reserves accumulated are typically starch. Thus these forms will usually give a typical black or deep purple color when treated with iodine.

A Individual cells of the following genera are perfectly round, or nearly so. The first does not form organized colonies. In the next two the colonies themselves tend to be round, and in the last, the colonies are triangular or irregular, and the cells bear long slender spines.

1 Chlorella cells are small and spherical to broadly elliptical. They have a single parietal chloroplast. This is a very large genus with an unknown number of similar appearing species, living in a great variety of habitats. Although often accumulating in great numbers, organized colonies are not formed.

a Chlorella ellipsoides is reported to be a common plankton form.

b Chlorella pyrenoidosa and Chlorella vulgaris are often found in organically enriched waters. Indeed a dominance of Chlorella species is considered in some places to be an indication that a sewage stabilization pond is functioning to maximum capacity.

c Chlorella pyrenoidosa is reported as a filter clogger in water treatment plants.

2 Sphaerocystis colonies are free floating and almost always with a perfectly spherical, homogeneous gelatinous envelope. Up to 32 spherical cells may be included. Sphaerocystis scheoeteri, the only species, is of wide occurrence in the plankton of lakes and reservoirs.

3 Coelastrum forms coenobial* colonies of up to 128 cells. Generally spherical or polygonal in shape--both cells and colony. Cells connected by protoplasmic processes of varying length. Coelastrum microporum is often reported in the plankton of water supplies. Not surrounded by gelatinous envelope as in Sphaerocystis.

4 Micractinium. The cells of this alga are spherical to broadly ellipsoidal and are usually united in irregular 4-celled coenobes. These in turn are almost always united with other coenobes to form multiple associations of up to 100 or more cells. The free face of

1 Including miscellaneous yellow-brown algae.

*A coenobe is a colony in which the number of cells does not increase during the life of the colony. It was established by the union of several independent swimming cells which simply stick together and increase in size.

each cell in a coenobe bears from one to seven very long slender setae or hairs.

Micractinium pusillum. This is a strictly planktonic genus.

B Individual cells of the following genera are elongate. In the first two they are relatively straight or irregular and pointed. The next two are also long and pointed, but bent into a tight "C" shape (one in a gelatinous envelope, one naked). The last one (Actinastrum) is long and straight, but with blunt ends, and with the cells of a coenobe attached at a point.

1 Ankistrodesmus cells are usually long and slender, tapering to sharp point at both ends. They may be straight, curved, or twisted into loose aggregations. Ankistrodesmus falcatus is often found in the plankton in water supplies and is considered to be one of the forms indicative of clean water.

2 Schroederia is a solitary, free floating alga. Cells are long and pointed at both ends. May be bent in various ways. Terminal points are continued as long slender spines which may be forked and bent back, or end as a plate. Of the three species reported in this country, Schroederia setigera has been reported in water supplies.

3 Selenastrum cells are pointed at both ends, and bent so that their tips approach each other. They tend to occur in groups of 4, 8, or 16, which may be associated with other groups to form masses of a hundred or more cells. There is no gelatinous envelope. Selenastrum gracile occurs in the plankton of water supplies.

4 Kirchneriella. The cells of this genus are generally relatively broad, tapering to a sharp or rounded point at each end, and the whole cell bent into a C-shape. They usually occur in groups of four to eight in a broad, homogeneous, gelatinous matrix. Kirchneriella lunaris is known principally from the plankton.

5 Actinastrum colonies or "coenobes" are composed of 4, 8, or 16 elongate cells that radiate in all directions from a common center.

Actinastrum is a widely distributed plankton organism. There are two species:

Actinastrum gracillimum and Actinastrum Hantzschii differ only in the sharpness of the taper toward the tips of the cells. The former has relatively little taper, and the latter, more.

C Cells of the following genera are associated in simple naked colonies. The first has elongate cells arranged with their long axes parallel (although some cells may be curved). The last two are flat plate-like coenobes. Crucigenia has four-celled coenobes while Pediastrum coenobes may be larger, appear plate-like, and are much more ornate.

1 Scenedesmus is a flat plate of elliptical to double ended pointed cells arranged with their long axes parallel. Coenobes consist of up to 32, but usually 4 to 8 cells. The number of cells in a coenobe may vary from mother to daughter colony. The appearance of cells may vary considerably with the species.

a Scenedesmus bijuga, S. dimorphus, and S. quadricauda are common planktonic forms.

b Scenedesmus quadricauda is also common in organically enriched water, and may become dominant.

c Scenedemus abundans is reported to impart a grassy odor to drinking water.

2 Crucigenia forms free floating four-celled coenobes that are solitary or joined to one another to form plate-like multiple coenobes of 16 or more cells. The cells may be elliptical, triangular, trapezoidal, or semi-circular in surface view. Crucigenia quadrata is a species often reported from water supplies.

3 Pediastrum. Colonies are free floating with up to 128 polygonal cells arranged in a single plane. There may or may not be open spaces between the cells. The exact arrangement of the cells seems to depend largely on the chance distribution of the original motile swarming zoaspores at the time the coenobe was formed. Peripheral cells may differ in shape from interior cells.

a Pediastrum boryanum and P. duplex are frequently found in the plankton, but seldom dominate.

b Pediastrum tetras has been reported to impart a grassy odor to water supplies.

D Cells of the following Genera are slightly elongated.

1 Oocystis. The cells of Oocystis may be solitary, or up to 16 cells may be surrounded by a partially gellatinized and greatly expanded mother cell wall. Cells may be ellipsoidal or almost cylindrical, cell wall thin, no spines or other ornamentation. Oocystis borgei, for example, is of frequent occurrence in the plankton.

2 Dimorphococcus cells are arranged in groups of four, and these tetrads are united to one another in irregularly shaped free floating colonies by the branching remains of old mother-cell walls. Two shapes of cell are normally found in each tetrad (hence the name), two longer ovate cells end to end, and a pair of slightly shorter, C-shaped cells on either side. Dimorphococcus lunatus is a widely distributed plankton organism, sometimes reported in considerable numbers.

E A distinctive group of green algae characterized by a median constriction dividing the cell into two geometrically similar halves is known generally as the "desmids." (Closterium and Penium do not have this construction). Each half of the cell is known as a "semicell." The nucleus lies in the "isthmus." Extremes of ornamentation and structural variety exist. Most are unicellular, but a few are filamentous or have the cells associated in shapeless colonies. They are found sparingly in the plankton almost everywhere, but predominate in acid waters.

1 Closterium is one of the exceptional genera without a median constriction. The cells are elongate, attenuated toward the tips but not sharply pointed, usually somewhat bent.

a Closterium aciculare is a planktonic species.

b Closterium moniliforme is reported as a filter clogging organism.

2 Cosmarium is a large, poorly defined genus of over 280 species, many of which apparently intergrade with other genera such as Staurastrum. In general, it can be said that Cosmarium species are relatively small, with a length only slightly greater than the width, and with a deep median constriction. Shapes of the semicells may vary greatly. Although shallow surface ornamentation may occur, long spines do not occur.

a Cosmarium botrytis is reported in plankton from water supply reservoirs.

b Cosmarium portianum is said to impart a grassy odor to water.

c Other species have been reported to be sufficiently resistant to chlorine to penetrate rapid sand filters and occur in distribution systems in considerable numbers.

3 Micrasterias is relatively common, ornate.

4 Euastrum cells tend to be at least twice as long as broad, with a deeply constricted isthmus, and a dip or incision at the tip of each semicell. The cell wall may be smooth, granulate, or spined.

Euastrum oblongum is reported as a planktonic species from water reservoirs. It has also been noted as intolerant of pollution, and hence an indicator of clean water.

5 Staurastrum is the commonest of the desmids in the plankton of fresh waters, the genus contains upwards of 245 species in the United States alone. Intergradation with other genera such as Cosmarium make it a difficult group to define. Most of the species are radially symmetrical, and almost all have a deeply constricted isthmus. The cell wall may be smooth, ornamented, or spined in a variety of ways. Relatively long truncated processes extending from the cell body in symmetrical patterns are common.

a Staurastrum polymorphum is a typical planktonic form.

b Staurastrum punctulatum is reported as an indicator of clean water.

c Staurastrum paradoxicum causes a grassy odor in water.

III A type of "green" alga known as "golden green" (Xanthophyceae) is represented in the plankton by two genera. In these algae there is a predominance of yellow over green pigments, hence frequently imparting a yellowish or golden tint to the cell. Reserve food material is stored as oil and leucosin, rather than as starch, hence giving a negative test with iodine in most cases.

A Botryococcus braunii is a widely distributed plankton alga, though it is rarely abundant.

1 The plant body is a free floating colony of indefinite shape, with a cartilaginous and hyaline or orange-colored envelope, surface greatly wrinkled and folded.

2 Individual cells lie close together, in several aggregates connected in reticular fashion by strands of the colonial envelope.

3 The envelope structure tends to obscure cell structure. Considerable deep orange colored oil may collect within the envelope, outside of the cells, obscuring cell structure.

B Ophiocytium capitatum like Botryococcus, is widely distributed, but seldom abundant.

1 Both ends of cylindrical cell are rounded, with a sharp spine extending therefrom.

2 Many nuclei and several chloroplasts are present.

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DIATOMS

I GENERAL CHARACTERISTICS

A Diatoms have cells of very rigid form due to the presence of silica in the wall. They contain a brown pigment in addition to the chlorophyll. Their walls are ornamented with markings which have a specific pattern for each kind.

1 The cells often are isolated but others are in filaments or other shapes of colonies.

2 The protoplast contains normal cell parts, the most conspicuous being the plastids. No starch is present.

B Cell shapes include the elongate ("pennate") and the short cylindric ("centric") one view of which is circular.

1 Pennate diatoms may be symmetrical, transversely unsymetrical, or longitudinally unsymmetrical.

C Wall is formed like a box with a flanged cover fitting over it.

1 "Valve" view is that of the top of the cover or the bottom of the box.

2 "Girdle" view is that of the side where flange of cover fits over the box.

3 End view is also possible for pennate types.

D Cell markings include·

1 Raphe or false raphe extending longitudinally.

2 Striations which are lines of pores extending from the area of the raphe to the margin. Coarse ones are "costae".

3 Nodules which may be terminal and central.

4 Internal shelves ("septae") extending longitudinally or transversely.

II REPRODUCTION

A The common method is by cell division. Two new half cells are formed between the halves of the parent cell.

B Auxospores and gametes may also be formed.

III EXAMPLES OF COMMON DIATOMS.

A Pennate, symmetrical

Navicula
Pinnularia
Synedra
Nitzschia
Diatoma
Fragilaria
Tabellaria
Cocconeis

B Pennate, unsymmetrical

Gomphonema
Surirella
Cymbella
Achnanthes
Asterionella
Meridion

C Centric

Cyclotella
Stephanodiscus
Melosira

IV Habitats include fresh and salt water. Both planktonic and attached forms occur, the latter often are broken loose. They may be attached by stalks or by their slimy surface.

A Many diatoms are more abundant in late autumn, winter, and early spring than in the warmer season.

B The walls of dead diatoms generally remain undecomposed and may be common in water. Many deposits of fossil diatoms exist.

V Importance of diatoms is in part due to their great abundance and their rigid walls.

A They are the most important group of organisms causing clogging of sand filters.

B Several produce tastes and odors in water, including the obnoxious fishy flavor.

C Mats of growth may cause floors or steps of swimming pools to be slippery.

D They may be significant in determining water quality in relation to pollution.

E They release oxygen into the water.

VI Classification. There are several thousand species of diatoms. Only the most common of the freshwater forms are considered here.

A Centrales Group

1 Representative genera.

Cyclotella
Stephanodiscus
Melosira
Rhizosolenia
Biddulphia

B Pennals Group

1 Fragilarineae. The false raphe group.

Representative genera

Tabellaria
Meridion
Diatoma
Fragilaria
Synedra
Asterionella

2 Achnanthineae. Group with cells having one false and one true raphe.

a Representative genera

Cocconeis
Achnanthes

3 Naviculineae. True raphe group with raphe in center of valve.

a Representative genera.

Navicula
Pinnularia
Stauroneis
Pleurosigma
Amphiprora
Gomphonema
Cymbella
Epithemia

4 Surirellineae. True raphe group with raphe near one side of valve.

a Representative genera.

Nitzschia
Cymatopleura
Surirella
Campylodiscus

VII IDENTIFICATION OF DIATOMS

A Some genera are easily recognized by their distinctive shape.

B Many genera and most species can be determined only after diatoms are freed of their contents and observed under the high magnification of an oil immersion lens of the compound microscope.

C Contents of the cell are generally not used in identification. Only the characteristics of the wall are used.

D For identification of genera, most important features include.

1 Cell shape, and form of colony

2 Raphe and false raphe

3 Striations

4 Septa

E For identification of species, measurements involving the number of striae per 10 microns, the direction of the striae and many other characteristics may be needed.

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II KEY TO ALGAE OF IMPORTANCE IN WATER POLLUTION

1	Plant a tube, thread, strand, ribbon, or membrane, frequently visible to the unaided eye	2
1'	Plants of microscopic cells which are isolated or in irregular, spherical, or microscopic clusters, cells not grouped into threads	123
2 (1)	Plant a tube, strand, ribbon, thread, or membrane composed of cells	3
2'	Plant a branching tube with continuous protoplasm, not divided into cells	120
3 (2)	Plant a tube, strand, ribbon, thread, or a mat of threads	4
3'	Plant a membrane of cells one cell thick (and 2 or more cells wide)	116
4 (3)	Cells in isolated or clustered threads or ribbons which are only one cell thick or wide	5
4'	Cells in a tube, strand, or thread all (or a part) of which is more than one cell thick or wide	108
5 (4)	Heterocysts present	6
5'	Heterocysts absent	23
6 (5)	Threads gradually narrowed to a point at one end	7
6'	Threads same width throughout	12
7 (6)	Threads as radii, in a gelatinous bead or mass	8
7'	Threads not in a gelatinous bead or mass	11
8 (7)	Spore (akinete) present, adjacent to the terminal heterocyst (Gloeotrichia)	9
8'	No spore (akinete) present (Rivularia)	10
9 (8)	Gelatinous colony a smooth bead	Gloeotrichia echinulata
9'	Gelatinous colony irregular	Gloeotrichia natans
10 (8')	Cells near the narrow end as long as wide	Rivularia dura
10'	Cells near the narrow end twice as long as wide	Rivularia haematites
11 (7')	Cells adjacent to heterocyst wider than heterocyst	Calothrix braunii
11'	Cells adjacent to heterocyst narrower than heterocyst	Calothrix parietina
12 (6')	Branching present	13
12'	Branching absent	14
13 (12)	Branches in pairs	Scytonema tolypothricoides
13'	Branches arising singly .	Tolypothrix tenuis
14 (12')	Heterocyst terminal only (Cyclindrospermum)	15
14'	Hetrocysts intercalary (within the filament)	16
15 (14)	Heterocyst round	Cylindrospermum muscicola
15'	Heterocyst elongate	Cylindrospermum stagnale
16 (14')	Threads encased in a gelatinous bead or mass	17
16'	Threads not encased in a definite gelatinous mass	18
17 (16)	Heterocysts and vegetative cells rounded	Nostoc pruniforme
17'	Heterocysts and vegetative cells oblong	Nostoc carneum
18 (16')	Heterocysts and vegetative cells shorter than the thread width	Nodularia spumigena
18'	Heterocysts and vegetative cells not shorter than the thread width	19
19 (18')	Heterocysts rounded (Anabaena).	20
19'	Heterocysts clindric	Aphanizomenon flos-aquae
20 (19)	Cells elongate, depressed in the middle, heterocysts rare	Anabaena constricta
20'	Cells rounded, heterocysts common	21
21 (20')	Heterocysts with lateral extensions	Anabaena planctonica
21'	Heterocysts without lateral extensions	22

22 (21')	Threads 4-8μ wide	Anabaena flos-aquae
22'	Threads 8-14μ wide	Anabaena circinalis
23 (5')	Branching absent	24
23'	Branching (including "false" branching) present	84
24 (23)	Cell pigments distributed throughout the protoplasm	25
24'	Cell pigments limited to plastids	49
25 (23)	Threads short and formed as an even spiral	285
25'	Threads very long and not forming an even spiral	26
26 (25')	Several parallel threads of cells in one common sheath	Microcoleus subtorulosus
26'	One thread per sheath if present	27
27 (26')	Sheath or gelatinous matrix present	28
27'	No sheath nor gelatinous matrix apparent (Oscillatoria)	35
28 (27)	Sheath distinct no gelatinous matrix between threads (Lyngbya)	29
28'	Sheath indistinct or absent, threads interwoven with gelatinous matrix between (Phormidium) .	32
29 (28)	Cells rounded	Lyngbya ocracea
29'	Cells short cylindric	30
30 (29')	Threads in part forming spirals	Lyngbya lagerheimii
30'	Threads straight or bent but not in spirals	31
31 (30')	Maximum cell length 3.5μ, sheath thin	Lyngbya digueti
31'	Maximum cell length 6.5μ, sheath thick	Lyngbya versicolor
32 (28')	Ends of some threads with a rounded swollen "cap" cell	33
32'	Ends of all threads without a "cap" cell	34
33 (32)	End of thread (with "cap") abruptly bent	Phormidium uncinatum
33'	End of thread (with "cap") straight	Phormidium autumnale
34 (32')	Threads 3-5μ in width	Phormidium inundatum
34'	Threads 5-12μ in width	Phormidium retzii
35 (27')	Cells very short, generally less than 1/3 the thread diameter	36
35'	Cells generally 1/2 as long to longer than the thread diameter	39
36 (35)	Cross walls constricted	Oscillatoria ornata
36'	Cross walls not constricted	37
37 (36')	Ends of thread, if mature, curved	38
37'	Ends of thread straight	Oscillatoria limosa
38 (37)	Threads 10-14μ thick	Oscillatoria curviceps
38'	Threads 16-60μ thick	Oscillatoria princeps
39 (35')	Threads appearing red to purplish	Oscillatoria rubescens
39'	Threads yellow-green to blue-green	40
40 (39')	Threads yellow-green	41
40'	Threads blue-green	43
41 (40)	Cells 4-7 times as long as the thread diameter	Oscillatoria putrida
41'	Cells less than 4 times as long as the thread diameter	42
42 (41')	Prominent granules ("pseudovacuoles") in center of each cell	Oscillatoria lauterbornii
42'	No prominent granules in center of cells	Oscillatoria chlorina
43 (40')	Cells 1/2-2 times as long as the thread diameter	44
43'	Cells 2-3 times as long as the thread diameter	48
44 (43)	Cell walls between cells thick and transparent	Oscillatoria pseudogeminata
44'	Cell walls thin, appearing as a dark line	45

45 (44')	Ends of thread straight	Oscillatoria agardhii
45'	Ends of mature threads curved	46
46 (45')	Prominent granules present especially at both ends of each cell	Oscillatoria tenuis
46'	Cells without prominent granules	47
47 (46')	Cross walls constricted	Oscillatoria chalybea
47'	Cross walls not constricted	Oscillatoria formosa
48 (43')	End of thread long tapering	Oscillatoria splendida
48'	End of thread not tapering	Oscillatoria amphibia
49 (24')	Cells separate from one another and enclosed in a tube (Cymbella)	251'
49'	Cells attached to one another as a thread or ribbon	50
50 (49')	Cells separating readily into discs or short cylinders their circular face showing radial markings .	233
50'	Cells either not separating readily, or if so, no circular end wall with radial markings	51
51 (50')	Cells in a ribbon attached side by side or by their corners	52
51'	Cells in a thread, attached end to end	56
52 (51)	Numerous regularly spaced markings in the cell wall	53
52'	Numerous markings in the cell wall absent (Scenedesmus)	128
53 (52)	Wall markings of two types one coarse, one fine	185
53'	Wall markings all fine (Fragilaria)	54
54 (53')	Cells attached at middle portion only	*Fragilaria crotonensis*
54'	Cells attached along entire length	55
55 (54')	Cell length 25-100μ	Fragilaria capucina
55'	Cell length 7-25μ	Fragilaria construens
56 (51')	Plastid in the form of a spiral band (Spirogyra)	57
56'	Plastid not a spiral band	61
57 (56)	One plastid per cell	58
57'	Two or more plastids per cell	60
58 (57)	Threads 18-26μ wide	Spirogyra communis
58'	Threads 28-50μ wide	59
59 (58')	Threads 28-40μ wide	Spirogyra varians
59'	Threads 40-50μ wide	Spirogyra porticalis
60 (57')	Threads 30-45μ wide, 3-4 plastids per cell	Spirogyra fluviatilis
60'	Threads 50-80μ wide, 5-8 plastids per cell	Spirogyra majuscula
61 (56')	Plastids two per cell	62
61'	Plastids either one or more than two per cell	66
62 (61)	Cells with knobs or granules on the wall	63
62'	Cells with a smooth outer wall	64
63 (62)	Each cell with two central knobs on the wall	Desmidium grevillii
63'	Each cell with a ring of granules near one end	Hyalotheca mucosa
64(62')	Cells dense green, each plastid reaching to the wall	Zygnema sterile
64'	Cells light green, plastids not completely filling the cell	65
65 (64')	Width of thread 26-32μ, maximum cell length 60μ	Zygnema insigne
65'	Width of thread 30-36μ, maximum cell length 120μ	Zygnema pectinatum
66 (61')	Plastid a wide ribbon, passing through the cell axis (Mougeotia) .	67
66'	Plastid or plastids close to the cell wall (parietal)	69

67 (66)	Threads with occasional "knee-joint" bends	Mougeotia genuflexa
67'	Threads straight	68
68 (67')	Threads 19-24μ wide, pyrenoids 4-16 per cell	Mougeotia sphaerocarpa
68'	Threads 20-34μ wide, pyrenoids 4-10 per cell	Mougeotia scalaris
69 (66')	Occasional cells with one to several transverse wall lines near one end (Oedogonium)	70
69'	Occasional terminal transverse wall lines not present	73
70 (69)	Thread diameter less than 24μ	71
70'	Thread diameter 25μ or more	72
71 (70)	Thread diameter 9-14μ	Oedogonium suecicum
71'	Thread diameter 14-23μ	Oedogonium boscii
72 (70)	Dwarf male plants attached to normal thread, when reproducing	Oedogonium idioandrosporum
72'	No dwarf male plants produced	Oedogonium grande
73 (69')	Cells with one plastid which has a smooth surface	74
73'	Cells with several plastids or with one nodular plastid	78
74 (73)	Cells with rounded ends	Stichococcus bacillaris
74'	Cells with flat ends (Ulothrix)	75
75 (74')	Threads 10μ or less in diameter	76
75'	Threads more than 10μ in diameter	77
76 (75)	Threads 5-6μ in diameter	Ulothrix variabilis
76'	Threads 6-10μ in diameter	Ulothrix tenerrima
77 (75')	Threads 11-17μ in diameter	Ulothrix aequalis
77'	Threads 20-60μ in diameter	Ulothrix Zonata
78 (73')	Iodine test for starch positive, one nodular plastid per cell	79
78'	Iodine test for starch negative, several plastids per cell	80
79 (78)	Thread when broken, forming "H" shape segments	Microspora amoena
79'	Thread when fragmented, separating irregularly or between cells (Rhizoclonium)	100
80 (78')	Side walls of cells straight, not bulging A pattern of fine lines or dots present in the wall but often indistinct (Melosira)	81
80'	Side walls of cells slightly bulging Pattern of wall markings not present (Tribonema)	83
81 (80)	Spine-like teeth at margin of end walls	82
81'	No spine-like teeth present	Melosira varians
82 (81)	Wall with fine granules, arranged obliquely	Melosira crenulata
82'	Wall with coarse granules, arranged parallel to sides	Melosira granulata
83 (80')	Plastids 2-4 per cell	Tribonema minus
83'	Plastids more than 4 per cell	Tribonema bombycinum
84 (23')	Plastids present, branching "true"	85
84'	Plastids absent, branching "false"	Plectonema tomasiniana
85 (84)	Branches reconnected, forming a net	Hydrodictyon reticulatum
85'	Branches not forming a distinct net	86
86 (85')	Each cell in a conical sheath open at the broad end (Dinobryon)	87
86'	No conical sheath around each cell	90
87 (86)	Branches diverging, often almost at a right angle	Dinobryon divergens
87'	Branches compace often almost parallel	88
88 (87')	Narrow end of sheath sharp pointed	89
88'	Narrow end of sheath blunt pointed	Dinobryon sertularia

89 (88)	Narrow end drawn out into a stalk	Dinobryon stipitatum
89'	Narrow end diverging at the base	Dinobryon sociale
90 (86')	Short branches on the main thread in whorls of 4 or more (Nitella)	91
90'	Branching commonly single or in pairs	92
91 (90)	Short branches on the main thread rebranched once	Nitella flexilis
91'	Short branches on the main thread rebranched two to four times	Nitella gracilis
92 (90')	Terminal cell each with a colorless spine having an abruptly swollen base (Bulbochaete)	93
92'	No terminal spines with abruptly swollen bases	94
93	Vegetative cells 20-48µ long	Bulbochaete mirabilis
93'	Vegetative cells 48-88µ long	Bulbochaete insignis
94 (92')	Cells red, brown, or violet	Audouinella violacea
94'	Cells green	95
95 (94')	Threads enclosed in a gelatinous bead or mass	96
95'	Threads not surrounded by a gelatinous mass	99
96 (95)	Abrupt change in width from main thread to branches (Draparnaldia)	97
96'	Gradual change in width from main thread to branches (Chaetophora)	98
97 (96)	Branches (from the main thread) with a central, main axis	Draparnaldia plumosa
97'	Branches diverging and with no central main axis	Draparnaldia glomerata
98 (96')	End cells long-pointed, with colorless tips	Chaetophora attenuata
98'	End cells abruptly pointed, mostly without long colorless tips	Chaetophora elegans
99 (95')	Light and dense dark cells intermingled in the thread	Pithophora oedogogonia
99'	Most of the cells essentially alike in density	100
100 (99')	Branches few in number, and short colorless	Rhizoclonium hieroglyphicum
100'	Branches numerous and green	101
101 (100')	Terminal attenuation gradual, involving two or more cells (Stigeoclonium)	102
101'	Terminal attenuation absent or abrupt, involving only one cell (Cladophora)	104
102 (101)	Branches frequently in pairs	103
102'	Branches mostly single	Stigeoclonium stagnatile
103 (102)	Cells in main thread 1-2 times as long as wide	Stigeoclonium lubricum
103'	Cells in main thread 2-3 times as long as wide	Stigeoclonium tenue
104 (101')	Branching often appearing forked, or in threes	Cladophora aegagropila
104'	Branches distinctly lateral	105
105 (104')	Branches forming acute angle with main thread, thus forming clusters	Cladophora glomerata
105'	Branches forming wide angles with the main thread	106
106 (105')	Threads crooked and bent	Cladophora fracta
106'	Threads straight	107
107 (106')	Branches few, seldom rebranching	Cladophora insignis
107'	Branches numerous, often rebranching	Cladophora crispata
108 (4')	Plant or tube with a tight surface layer of cells and with regularly spaced swellings (nodes)	Lemanea annulata
108'	Plant not a tube that has both a tight layer of surface cells and nodes	109
109 (108')	Cells spherical and loosely arranged in a gelatinous matrix	Tetraspora gelatinosa
109'	Cells not as loosely arranged spheres	110
110 (109')	Plants branch	111
110'	Plants not branched	Schizomeris leibleinii
111 (110)	Clustered branching	112
111'	Branches single	115

112 (111)	Threads embedded in gelatinous matrix (Batrachospermum)	113
112'	No gelantinous matrix (Chara)	114
113 (112)	Nodal masses of branches touching one another	Batrachospermum vagum
113'	Nodal masses of branches separated by a narrow space	Batrachospermum moniliforme
114 (112')	Short branches with 2 naked cells at the tip	Chara globularis
114'	Short branches with 3-4 naked cells at the tip	Chara vulgaris
115 (111')	Heterocysts present, plastids absent	Stigonema minutum
115'	Heterocysts absent, plastids present	Compsopogon coeruleus
116 (3')	Red eye spot and two flagella present for each cell	125
116'	No eye spots nor flagella present	117
117 (116')	Round to oval cells, held together by a flat gelatinous matrix (Agmenellum)	131
117'	Cells not round and not enclosed in a gelatinous matrix	118
118 (117')	Cells regularly arranged to an unattached disc Number of cells 2, 4, 8, 16, 32, 64, or 128	133'
118'	Cells numerous, membrane attached on one surface	119
119 (118')	Long hairs extending from upper surface of cells	Chaetopeltis megalocystis
119'	No hairs extending from cell surfaces	Hildenbrandia rivularis
120 (2')	Constriction at the base of every branch	Dichotomosiphon tuberosus
120'	No constrictions present in the tube (Vaucheria)	121
121 (120')	Egg sac attached directly, without a stalk, to the main vegetative tube	Vaucheria sessilis
121'	Egg sac attached to an abrupt, short, side branch	122
122 (121')	One egg sac per branch	Vaucheria terrestris
122'	Two or more egg sacs per branch	Vaucheria geminata
123 (1')	Cells in colonies generally of a definite form or arrangement	124
123'	Cells isolated, in pairs or in loose, irregular aggregates	173
124 (123)	Cells with many transverse rows of markings on the wall	185
124'	Cells without transverse rows of markings	125
125 (124')	Cells arranged as a layer one cell thick	126
125'	Cell cluster more than one cell thick and not a flat plate	137
126 (125)	Red eye spot and two flagella present for each cell	Gonium pectorale
126'	No red eye spots nor flagella present	127
127 (126')	Cells elongate, united side by side in 1 or 2 rows (Scenedesmus)	128
127'	Cells about as long as wide	131
128 (127)	Middle cells without spines but with pointed ends	Scenedesmus dimorphus
128'	Middle cells with rounded ends	129
129 (128'')	Terminal cells with spines	130
129'	Terminal cells without spines	Scenedesmus bijuga
130 (129)	Terminal cells with two spines each	Scenedesmus quadricauda
130'	Terminal cells with three or more spines each	Scenedesmus abundans
131 (117)	Cells in regular rows, immersed in colorless matrix (Agmenellum quadriduplicatum)	132
131'	Cells not immersed in colorless matrix	133
132 (131)	Cell diameter 1 3 to 2 2μ	Agmenellum quadriduplicatum , tenuissima type
132'	Cell diameter 3-5μ	Agmenellum quadriduplicatum, glauca type
133 (131')	Cells without spines, projections, or incisions	Crucigenia quadrata
133'	Cells with spines, projections, or incisions	134

134 (133')	Cells rounded	Micractinium pusillum
134'	Cells angular (Pediastrum)	135
135 (134')	Numerous spaces between cells	Pediastrum duplex
135'	Cells fitted tightly together	136
136 (135')	Cell incisions deep and narrow	Pediastrum tetras
136'	Cell incisions shallow and wide	Pediastrum boryanum
137 (125')	Cells sharp-pointed at both ends, often arcuate	138
137'	Cells not sharp-pointed at both ends, not arcuate	141
138 (137)	Cells embedded in a gelatinous matrix	Kirchneriella lunaris
138'	Cells not embedded in a gelatinous matrix	139
139 (138')	Cells all arcuate, arranged back to back	Selenastrum gracile
139'	Cells straight or bent in various ways, loosely arranged or twisted together (Ankistrodesmus)	140
140 (139')	Cells bent	Ankistrodesmus falcatus
140'	Cells straight	Ankistrodesmus falcatus var acicularis
141 (137')	Flagella present eye spots often present	142
141'	No flagella nor eye spots present	152
142 (141)	Each cell in a conical sheath open at the wide end (Dinobryon)	86
142'	Individual cells not in conical sheaths	143
143 (142')	Each cell with 1-2 long straight rods extending	Chrysosphaerella longispina
143'	No long straight rods extending from the cells	144
144 (143')	Cells touching one another in a dense colony	145
144'	Cells embedded separately in a colorless matrix	149
145 (144)	Cells arranged radially, facing outward	146
145'	Cells all facing in one direction	147
146 (145)	Plastids brown, eye spot absent	Synura uvella
146'	Plastids green, eye spot present in each cell	Pandorina morum
147 (145')	Each cell with 4 flagella	Spondylomorum quaternarium
147'	Each cell with 2 flagella (Pyrobotrys)	148
148 (147')	Eye spot in the wider (anterior) end of the cell	Pyrobotrys stellata
148'	Eye spot in the narrower (posterior) end of the cell	Pyrobotrys gracilis
149 (144')	Plastids brown	Uroglenopsis americana
149'	Plastids green	150
150 (149')	Cells 16, 32, or 64 per colony	Eudorina elegans
150'	Cells more than 100 per colony	151
151 (150')	Colony spherical, each cell with an eye spot.	Volvox aureus
151'	Colony tubular or irregular no eye spots (Tetraspora)	109
152 (141')	Elongate cells, attached together at one end, arranged radially (Actinastrum)	153
152'	Cells not elongate, often spherical	154
153 (152)	Cells cylindric	Actinastrum gracillimum
153'	Cells distinctly bulging	Actinastrum hantzschii
154 (152')	Plastids present	155
154'	Plastids absent, pigment throughout each protoplast	168
155 (154)	Colonies, including the outer matrix, orange to red-brown	Botryococcus braunii
155'	Matrix if any, not bright colored, cell plastids green	156

156 (155')	Colonies round to oval	160
156'	Colonies not round, often irregular in form	157
157 (156')	Straight (flat) walls between adjacent cells (Phytoconis)	278
157'	Walls between neighboring cells rounded	158
158 (157')	Cells arranged as a surface layer in a large gelatinous tube (Tetraspora)	109
158'	Colony not a tube, cells in irregular pattern	159
159 (158')	Large cells more than twice the diameter of the small cells (Chlorococcum).	280'
159'	Large cells not more than twice the diameter of the small cells (Palmella)	281
160 (156)	Cells touching one another, tightly grouped	Coelastrum microporum
160'	Cells loosely grouped	161
161 (160')	Colorless threads extend from center of colony to cells	162
161'	No colorless threads attached to cells in colony.	164
162 (161)	Cells rounded or straight, oval (Dictyosphaerium)	163
162'	Cells elongate, some cells curved	Dimorphococcus lunatus
163 (162)	Cells rounded	Dictyosphaerium pulchellum
163'	Cells straight, oval	Dictyosphaerium ehrenbergianum
164 (161')	Cells rounded	165
164'	Cells oval	Oocystis borgei
165 (164)	One plastid per cell	166
165'	Two to four plastids per cell	Gloeococcus schroeteri
166 (165)	Outer matrix divided into layers (Gloeocystis)	167
166'	Outer matrix homogeneous	Sphaerocystis schroeteri
167 (166)	Colonies angular	Gloeocystis planctonica
167'	Colonies rounded	Gloeocystis gigas
168 (154')	Cells equidistant from center of colony (Gomphosphaeria)	169
168'	Cells irregularly distributed in the colony	172
169 (168)	Cells with pseudovacuoles	Gomphospaeria wichurae
169'	Cells without pseudovacuoles	170
170 (169')	Cells 2-4 µ in diameter (Gomphosphaeria lacustris)	171
170'	Cells ovate	Gomphosphaeria aponina
171 (170)	Cells spherical	Gomphosphaeria lacustris, kuetzingianum type
171'	Cells 4-15 in diameter	Gomphosphaeria lacustris, collinsii type
172 (168')	Cells ovid, division plane perpendicular to long axis (Coccochloris)	286
172'	Cells rounded, or division plane perpendicular to short axis (Anacystis)	286'
173 (123')	Cells with an abrupt median transverse groove or incision	174
173'	Cells without an abrupt transverse median groove or incision	184
174 (173)	Cells brown, flagella present (armored flagellates)	175
174'	Cells green, no flagella (desmids)	178
175 (174)	Cell with 3 or more long horns	Ceratium hirundinella
175'	Cell without more than 2 horns	176
176 (175')	Cell wall of very thin smooth plates	Glenodinium palustre
176'	Cell wall of very thick rough plates (Peridinium)	177
177 (176')	Ends of cell pointed	Peridinium wisconsinense
177'	Ends of cell rounded	Peridinium cinctum
178 (174')	Margin of cell with sharp pointed, deeply cut lobes or long spikes	179
178'	Lobes, if present, with rounded ends	182

179 (178)	Median incision narrow, linear	*Micrasterias truncata*
179'	Median incision wide, "V" or "U" shaped (*Staurastrum*)	180
180 (179)	Margin of cell with long spikes	*Staurastrum paradoxum*
180'	Margin of cell without long spikes	181
181 (180'')	Ends of lobes with short spines	*Staurastrum polymorphum*
181'	Ends of lobes without spines	*Staurastrum punctulatum*
182 (178')	Length of cell about double the width	*Euastrum oblongum*
182'	Length of cell one to one and one-half times the width (*Cosmarium*)	183
183 (182')	Median incision narrow linear	*Cosmarium botrytis*
183'	Median incision wide, "U" shaped	*Cosmarium portianum*
184 (173')	Cells triangular	*Tetraedron muticum*
184'	Cells not triangular	185
185 (124)	Cells with one end distinctly different from the other	186
185'	Cells with both ends essentially alike	225
186 (185)	Numerous transverse (not spiral) regularly spaced wall markings present (diatoms)	187
186'	No transverse regularly spaced wall markings	193
187 (186)	Cells curved (bent) in girdle view	*Rhoicosphenia curvata*
187'	Cells not curved in girdle view	188
188 (187')	Cells with both fine and coarse transverse lines	*Meridion circulare*
188'	Cells with transverse lines all alike in thickness	189
189 (188')	Cells essentially linear to rectangular, one terminal swelling larger than the other (*Asterionella*)	190
189'	Cells wedge-shaped, margins sometimes wavy (*Gomphonema*)	191
190 (189)	Larger terminal swelling 1-1/2 to 2 times wider than the other	*Asterionella formosa*
190'	Larger terminal swelling less than 1-1/2 times wider than the other	*Asterionella gracillima*
191 (189')	Narrow end enlarged in valve view	*Gomphonema geminatum*
191'	Narrow end not enlarged in valve view	192
192 (191')	Tip of broad end about as wide as tip of narrow end in valve view	*Gomphonema parvulum*
192'	Tip of broad end much wider than tip of narrow end in valve view	*Gomphonema olivaceum*
193 (186')	Spine present at each end of cell	*Schroederia setigera*
193'	No spine on both ends of cell	194
194 (193')	Pigments in one or more plastids	195
194'	No plastid, pigments throughout the protoplast	*Entophysalis lemaniae*
195 (194)	Cells in a conical sheath (*Dinobryon*)	86
195'	Cells not in a conical sheath	196
196 (195')	Cell covered with scales and long spines	*Mallomonas caudata*
196'	Cells not covered with scales and long spines	197
197 (196')	Protoplasts separated by a space from a rigid sheath (lorica)	198
197'	No loose sheath around the cells	202
198 (197)	Cells compressed (flattened)	*Phacotus lenticularis*
198'	Cells not compressed	199
199 (198')	Lorica opaque, yellow to reddish or brown	*Trachelomonas crebea*
199'	Lorica transparent, colorless to brownish (*Chrysococcus*)	200
200 (199')	Outer membrane (lorica) oval	*Chrysococcus ovalis*
200'	Outer membrane (lorica) rounded	201

201 (200')	Lorica thickened around opening	Chrysococcus rufescens
201'	Lorica not thickened around opening	Chrysococcus major
202 (197')	Front end flattened diagonally	203
202'	Front end not flattened diagonally	206
203 (202)	Plastids bright blue-green (Chroomonas)	204
203'	Plastids brown, red, olive-green or yellowish	205
204 (203)	Cell pointed at one end	Chroomonas nordstetii
204'	Cell not pointed at one end	Chroomonas setoniensis
205 (203')	Gullet present, furrow absent	Cryptomonas erosa
205'	Furrow present, gullet absent	Rhodomonas lacustris
206 (202')	Plastids yellow-brown	Chromulina rosanoffi
206'	Plastids not yellow-brown, generally green	207
207 (206')	One plastid per cell	208
207'	Two to several plastids per cell	211
208 (207)	Cells tapering at each end	Chlorogonium euchlorum
208'	Cells rounded to oval	209
209 (208')	Two flagella per cell (Chlamydomonas)	210
209'	Four flagella per cell	Cateria multifilis
210 (209)	Pyrenoid angular, eye spot in front third of cell	Chlamydomonas reinhardi
210'	Pyrenoid circular, eye spot in middle third of cell	Chlamydomonas globosa
211 (207')	Two plastids per cell	Cryptoglena pigra
211'	Several plastids per cell	212
212 (211')	Cell compressed (flattened) (Phacus)	213
212'	Cell not compressed	214
213 (212)	Posterior spine short, bent	Phacus pleuronectes
213'	Posterior spine long, straight	Phacus longicauda
214 (212)	Cell margin rigid	215
214'	Cell margin flexible (Euglena)	217
215 (214)	Cell margin with spiral ridges	Phacus pyrum
215'	Cell margin without ridges, but may have spiral lines (Lepocinclis)	216
216 (215')	Posterior end with an abrupt, spine-like tip	Lepocinclis ovum
216'	Posterior end rounded	Lepocinclis texta
217 (214')	Green plastids hidden by a red pigment in the cell	Euglena sanguinea
217'	No red pigment except for the eye spot	218
218 (217')	Plastids at least 1/4 the length of the cell	219
218'	Plastids discoid or at least shorter than 1/4 the length of the cell	220
219 (218)	Plastids two per cell	Euglena agilis
219'	Plastids several per cell, often extending radiately from the center	Euglena viridis
220 (218')	Posterior end extending as an abrupt colorless spine	221
220'	Posterior end rounded or at least with no colorless spine	222
221 (220)	Spiral markings very prominent and granular	Euglena spirogyra
221'	Spiral markings fairly prominent, not granular	Euglena oxyuris
222 (220')	Small, length 35-55μ	Euglena gracilis
222'	Medium to large, length 65μ or more	223
223 (222')	Medium in size, length 65-200μ	224
223'	Large in size, length 250-290μ	Euglena ehrenbergii

224 (223)	Plastids with irregular edge, flagellum 2 times as long as cell	Euglena polymorpha
224'	Plastids with smooth edge, flagellum about 1/2 the length of the cell	Euglena deses
225 (185')	Cells distinctly bent (arcuate), with a spine or narrowing to a point at both ends	226
225'	Cells not arcuate	230
226 (225)	Vacuole with particles showing Brownian movement at each end of cell Cells not in clusters (Closterium)	227
226'	No terminal vacuoles Cells may be in clusters or colonies	228
227 (226)	Cell wide, width 30-70µ	Closterium moniliferum
227'	Cell long and narrow, width up to 5µ	Closterium aciculare
228 (226')	Cell with a narrow abrupt spine at each blunt end	Ophiocytium capitatum
228'	No blunt ended cells with abrupt terminal spines	229
229 (228')	Sharp pointed ends as separate colorless spines	193
229'	Sharp pointed ends as part of the green protoplast	137
230 (225)	One long spine at each end of cell	231
230'	No long terminal spines	232
231 (230)	Cell gradually narrowed to the spine	137
231'	Cell abruptly narrowed to the spine	Rhizosolenia gracilis
232	A regular pattern of fine lines or dots in the wall (diatoms)	233
232'	No regular pattern of fine lines or dots in the wall	276
233 (50, 232)	Cells circular in one (valve) view, short rectangular or square in other (girdle) view	234
233'	Cells not circular in one view	240
234 (233)	Valve surface with an inner and outer (marginal) pattern of striae (*Cyclotella*)	235
234'	Valve surface with one continuous pattern of striae (Stephanodiscus)	238
235 (234)	Cells small, 4-10µ in diameter	Cyclotella glomerata
235'	Cells medium to large, 10-80 in diameter	236
236 (235')	Outer half of valve with two types of lines, one long, one short	237
236'	Outer half of valve with radial lines all alike	Cyclotella meneghiniana
237 (236)	Outer valve zone constituting more than 1/2 the diameter	Cyclotella bodanica
237'	Outer valve zone constituting more than 1/2 the diameter	Cyclotella compta
238 (234')	Cell 4-25µ in diameter	239
238'	Cell 25-65µ in diameter	Stephanodiscus niagarae
239 (238)	Cell with two transverse bands, in girdle view	Stephanodiscus binderanus
239'	Cell without two transverse bands, in girdle view	Stephanodiscus hantzschii
240 (233')	Cells flat, oval (Cocconeis)	241
240'	Cells neither flat nor oval	242
241 (240)	Wall markings (striae) 18-20 in 10µ	Cocconeis pediculus
241'	Wall markings (striae) 23-25 in 10µ	Cocconeis placentula
242 (240')	Cell sigmoid in one view	243
242'	Cell not sigmoid in either round or point ended (valve) or square ended (girdle) surface view	244
243 (242)	Cell sigmoid in valve surface view	Gyrosigma attenuatum
243'	Cell sigmoid in square ended (girdle) surface view	Nitzschia acicularis
244 (242')	Cell longitudinally unsymmetrical in at least one view	245
244'	Cell longitudinally symmetrical	254
245 (244)	Cell wall with both fine and coarse transverse lines (striae and costae)	246
245'	Cell wall with fine transverse lines (striae) only	247

246 (245)	Valve face about as wide at middle as girdle face	*Epithemia turgida*
246'	Valve face 1/2 or less as wide at middle as girdle face	*Rhopalodia gibba*
247 (245)	Line of pores and raphe located at edge of valve face	248
247'	Raphe not at extreme edge of valve face	250
248 (247)	Raphe of each valve adjacent to the same girdle surface	*Hantzschia amphioxys*
248'	Raphe of each valve adjacent to different girdle surfaces (*Nitzschia*)	249
249 (248')	Cell 20-65μ long	*Nitzschia palea*
249'	Cell 70-180μ long	*Nitzschia linearis*
250 (247')	Cell longitudinally unsymmetrical in valve view	251
250'	Cell longitudinally unsymmetrical in girdle view	*Achnanthes microcephala*
251 (250)	Raphe bent toward one side at the middle	*Amphora ovalis*
251'	Raphe a smooth curve throughout (*Cymbella*)	252
252 (251') (246)	Cell only slightly unsymmetrical	*Cymbella cesati*
252'	Cell distinctly unsymmetrical	253
253 (252')	Striations distinctly cross lined, width 10-30μ	*Cymbella prostrata*
253'	Striations indistinctly cross lined, width 5-12μ	*Cymbella ventricosa*
254 (244')	Longitudinal line (raphe) and prominent marginal markings near both edges of valve	255
254'	No marginal longitudinal line (raphe) nor keel, raphe or pseudoraphe median	257
255 (254)	Margin of girdle face wavy	*Cymatopleura solea*
255'	Margin of girdle face straight (*Surirella*)	256
256 (255')	Cell width 8-23μ	*Surirella ovata*
256'	Cell width 40-60μ	*Surirella splendida*
257 (254)	Gridle face generally in view and with two or more prominent longitudinal lines In valve view, swollen central oval portion bounded by a line (*Tabellaria*)	258
257'	Girdle face with less than two prominent longitudinal lines In valve view, whole central portion not bounded by a line	259
258 (257)	Girdle face less than 1/4 as wide as long	*Tabellaria fenestrata*
258'	Girdle face more than 1/2 as wide as long	*Tabellaria flocculosa*
259 (257')	Valve face with both coarse and fine transverse lines	*Diatoma vulgare*
259'	Valve face with transverse lines, if visible, alike in thickness	260
260 (259')	Valve face naviculoid true raphe present	261
260'	Valve face linear to linear-lanceolate, true raphe absent	270
261 (260)	Valve face with wide transverse lines (costae) (*Pinnularia*)	262
261'	Valve face with thin transverse lines (striae)	263
262 (261)	Cell 5-6μ broad	*Pinnularia subcapitata*
262'	Cell 34-50μ broad	*Pinnularia nobilis*
263 (261')	Transverse lines (striae) absent across transverse axis of valve face	*Stauroneis phoenicenteron*
263'	Transverse lines (striae) present across transverse axis of valve face	264
264 (263')	Raphe strictly median (*Navicula*)	265
264'	Raphe located slightly to one side	252
265 (264)	Ends of valve face abruptly narrowed to a beak	*Navicula exigua* var *capitata*
265'	Ends of valve face gradually narrowed	266
266 (265')	Most of striations strictly transverse	*Navicula gracilis*
266'	Most of striations radial (oblique)	267

267 (266')	Striae distinctly composed of dots (punctae)	Navicula lanceolata
267'	Striae essentially as continuous lines	268
268 (267')	Central clear area on valve face rectangular	Navicula graciloides
268'	Central clear area on valve face oval	269
269 (268')	Cell length 29-40μ, ends slightly capitate	Navicula cryptocephala
269'	Cell length 30-120μ, ends not capitate	Navicula radiosa
270 (260')	Knob at one end larger than at the other (Asterionella)	189
270'	Terminal knobs if present equal in size (Synedra)	271
271 (270')	Clear space (pseudonodule) in central area	Synedra pulchella
271'	No pseudonodule in central area	272
272 (271')	Sides parallel in valve view, each end with an enlarged nodule	Synedra capitata
272'	Sides converging to the ends in valve view	273
273 (272')	Valve linear to lanceolate-linear, 8-12 striae per 10μ	Synedra ulna
273'	Valve narrowly linear-lanceolate, 12-18 striae per 10μ	.274
274	Valve 5-6μ wide	Synedra acus
274'	Valve 2-4μ wide	275
275 (274')	Cells up to 65 times as long as wide, central area absent to small oval	Synedra acus var radians
275'	Cells 90-120 times as long as wide, central area rectangular	Synedra acus var augustissima
276 (232')	Green to brown pigment in one or more plastids	277
276'	No plastids, blue and green pigments throughout protoplast	284
277 (276)	Cells long and narrow or flat	233
277'	Cells rounded	278
278 (277')	Straight, flat wall between adjacent cells in colonies	Phytoconis botryoides
278'	Rounded wall between adjacent cells in colonies	279
279 (278')	Cell either with 2 opposite wall knobs or colony of 2-4 cells surrounded by distinct membrane or both	164
279'	Cell without 2 wall knobs, colony not of 2-4 cells surrounded by distince membrane	280
280 (279')	Cells essentially similar in size within the colony	281
280'	Cells of very different sizes within the colony	Chlorococcum humicola
281 (159')	Cells embedded in an extensive gelatinous matrix	Palmella mucosa
281'	Cells with little or no gelatinous matrix around them (Chlorella)	282
282 (281')	Cells rounded	283
282'	Cells ellipsoidal to ovoid	Chlorella ellipsoidea
283 (282)	Cell 5-10μ in diameter, pyrenoid indistinct	Chlorella vulgaris
283'	Cell 3-5μ in diameter, pyrenoid distinct	Chlorella pyrenoidosa
284 (276')	Cell a spiral rod	285
284'	Cell not a spiral rod	286
285 (25)	Thread septate (with crosswalls)	Arthrospira jenneri
285'	Thread non-septate (without crosswalls)	Spirulina nordstedtii
286 (172) (284')	Cells dividing in a plane at right angles to the long axis	Coccochloris stagnina
286' (172')	Cells sperical or dividing in a plane parallel to the long axis (Anacystis)	287
287 (286')	Cell containing pseudovacuoles	Anacystis cyanea
287'	Cell not containing pseudovacuoles	288

288 (287')	Cell 2-6μ in diameter, sheath often colored	Anacystis montana
288'	Cell 6-50μ in diameter, sheath colorless	289
289 (288')	Cell 6-12μ in diameter, cells in colonies are mostly spherical	Anacystis thermalis
289'	Cell 12-50μ in diameter, cells in colonies are often angular	Anacystis dimidiata

AQUATIC MACROPHYTES

I INTRODUCTION

A This non-taxonomic description of aquatic plants includes not only the higher vascular plants, but also the larger algae like Chara and Lemanea and the mosses and liverworts. Many manuals on higher aquatic plants will also include the larger algae and lower plants for ecological convenience.

B Ecologically they are primary producers as are the phytoplankton. Their role in detrital cycles is considerable.

C The littoral zone of lakes and estuaries are often dominated by this group while in streams they fill important niches in both the riffles and shallow pool areas.

Water depth determines the adjustment of aquatic seed plants into three principal categories.

1 Surface or floating weeds generally grow in deeper water at the front of (oftentimes commingling with) the emersed weeds. The larger floating weeds are waterlilies that may be rooted in the mud of the bottom and bear large leaves that float upon the surface. Smaller types such as the duckweeds are free-floating.

2 Emersed weeds are those that occupy shallow water, are rooted in bottom mud, and support foliage, seeds and mature fruit one or more feet above the water surface. Cattails, rushes, and the marsh grasses are familiar examples.

3 Submersed aquatic growths often form a belt or zone of herbage farthest from shore. Except for those forms that dwell in quiet waters, they are rooted to the bottom. Depth varies considerably within this zone and may extend down to the limits of effective light penetration.

D In the long-term cycle of the change in the aquatic terrain there is a continuing tendency for the land to encroach upon shallow ponds and shallow areas of lakes, decrease their size, make them more shallow and eventually return them to dry land. Rooted and other aquatic vegetation plays a prominent role in this gradual process by

1 Invading shallow water areas through entrapment of particulate matter that is carried into lakes and ponds. The rooted vegetation will continue to spread as water areas become more shallow and the bottom mud provides suitable anchorage for roots. Mangrove growths in estuaries and along seashore areas have made swamps out of once open water areas.

2 Plants contribute also to the filling in of lakes and estuaries through both the precipitation of calcium carbonate and the accumulation of their remains upon death and decay, e.g., marl and peat moss.

E While these lake invasions by higher aquatic plants may sometimes be sufficiently rapid to be recognized by those who habitually use the lake, the common objections to rooted vegetation stem from their immediate interference with recreational use such as boating and encroachment on navigation channels and swimming beaches.

II MACROPHYTE GROUPS

A Division Algae

The green alga Chara (and related genera Nitella and Tolypella) may form "weed beds" (in lakes, ponds, streams, and estuaries) as extensive as higher plants and grossly similar. Other algae such as Lemanea may form dense mats in riffle areas of streams or springs. In marine waters the larger marine algae may form dense mats.

B Division Bryophyta (Liverworts and Mosses)

These are relatively small plants which lack flowers and conducting tissues (xylem and phloem).

1 The Life Cycle consists of two phases:

a The leafy green gametophyte which produces motile gametes.

b The sporophyte generation producing spores.

2 This is a freshwater group consisting of less than 50 genera. About one fourth of these are liverworts.

a The liverworts (Class Hepaticae) are small flattened green plants which may lack stems and leaves. Riccia is a slender, branched surface form growing in loose clusters of flat slender sprays. Fragments of these are often taken in plankton samples. Ricciocarpus is a notched oval form about one centimeter in diameter that is often found in the same environment.

b The true mosses (Class Musci) have distinct leaves and stems. Sphagnum forms extensive bogs in some areas. In cooler streams Fontinalis may occupy large areas. One species of the latter has been found resistant to a variety of complex wastes, including zinc.

c In swamps and along lake shores many species pass from an aquatic to terrestrial environment without showing specific morphological changes.

C Vascular Plants (Division Tracheophyta) include the aquatic fern or fern-like plants (Class Pteridophyta).

1 The aquatic lower tracheophytes are primarily freshwater. The Quillworts (Isoetaceae) are related to the club mosses and may form numerous rosettes on the silt-sand bottoms of lakes.

Division Tracheophyta - This division, like the preceding one (Bryophyta) has the life cycle of alternation of gametophyte and sporophyte. In this group, however, the sporophytes always become free living, independent plants with possession of conducting or vascular tissues (xylem and phloem). The aquatic lower Tracheophyta are primarily freshwater and only represented by a few species.

2 Included in the higher Tracheophyta are ferns (Class Filicinae) containing aquatic genera, Azolla, Salvinia, Marsilea, Ceratopteris.

a Azolla and Salvinia form floating masses sometimes interspersed with Duckweed. The former often forms a red carpet.

b Marsilea occupies the area between high and low water of stream and bayou margins.

c Ceratopteris is a floating form often of nuisance locally.

D Vascular Plants (Division Tracheophyta, Class Angiospermae)

1 Structural modification - all aquatic plants are characterized by certain specific morphological features relative to adaptation for the aquatic habitat.

a Many heterophyllous - submerged leaves may be narrow or incised. Surface leaves have simple compact blades.

b Root system only an anchor, with adsorption of dissolved salts taking place over entire plant surface, lacking root hairs.

c Stomata present only on upper side of leaves in floating plants.

d Chloroplasts located in the epidermis.

e Flower clusters may be above water and pollinated by wind or insects or pollen suspended in the water and pistils pollinated under water.

f Vegetative reproduction predominates. Small fragments (turions or winter buds) or root stocks (tubers) may be sufficient for propagation.

g A variety of plants are associated with the aquatic environment making a clear distinction between terrestrial and true hydrophytes difficult. Aquatic plants have been defined by Reid as "those whose seeds germinate in either the water phase or the substrate...and must spend part of their life cycle in water." This, therefore, includes submersed as well as emersed.

2 Classification - Around fifty families of Angiosperms are primarily aquatic, including thirty estuarine species.

3 Dicotyledons

a Family Nymphaceae - the water lilies, Nuphar and Nymphaea Watershield, Brasenia, and Cabomba.

b Family Ceratophyllaceae - includes one genera Ceratophyllum (Coontail or Hornwort). The stems which are entirely under water may sometimes be stiff with a coat of lime.

c Family Trapaceae - the water chestnut or caltrop, Trapa. The sharp spined fruits are a staple crop in parts of Asia. Colonies of connected rosettes of this plant quickly form surface floating mats which may impede navigation in the littoral region of lakes and in slow-moving rivers. Examples - Africa, Rumania, and Massachusetts.

d Family Haloragidaceae - includes two genera Proserpinica and Myriophyllum. The latter is found up to 15 parts per 1000 salinity and has been a serious nuisance in Chesapeake Bay and the Tennessee Valley. In the former area, a virus has reduced populations as much as 95 percent.

e Podostemaceae - primarily a tropical family which is highly adapted to life in rushing water, even in torrential areas over rocks worn smooth by the water and impenetrable by roots. This is made possible by haptera (attachment organs) which cement the plant to the substrate. Morphologically and ecologically they bear resemblances to certain attached algae. This group also shares the characteristics of other aquatic plants, but in addition possesses quantities of silica in the cell. Where the current in the stream is less vigorous the Podostemum will be replaced by mosses and algae. Podostemum communities are a challenge in quantitative sampling of the associated macroinvertebrates.

f Family Lentibulariaceae - Bladderworts, Utricularia, have small bladders which keep the stems afloat and also serve as traps for the capture of small organisms.

g Family Rhizophoraceae - Rhizophora mangle. The red mangrove.

h Family Combretaceae - Combretaceae - Laguncularia racemosa. The white mangrove.

i Family Verbenaceae - Avincennia nitida. The black mangrove
The three mangrove families listed above are widespread on both the East and West Coasts. They support a unique algal vegetation, mainly of certain red algal genera.

4 Monocotyledons

a Family Butomaceae - Butomus umbellatus is Eurasian in origin, and has spread rapidly in the St. Lawrence River Basin and around the Great Lakes.

b Family Hydrocharitaceae - includes the well known Elodea (= Anacharis) Hydrilla, Egeria, and Vallisneria plus two marine genera Halophila and Thalassia (T. testudinum, Turtle Grass.) The former fresh water genera also extend into brackish water.

c The water plantain family (Alismaceae) is a large group of emersed marsh plants and submersed aquatics. Sagittaria subulata is found in both fresh and estuarine areas. In the latter it matures without much change in shape or size of leaves (ribbon leaf form) and the plant and flowers may be under water at high tide.

d Family Zosteraceae

1) Zostera marina or Eel Grass is found on both coasts from Alaska to California and Hudson Bay to North Carolina. In the early thirties, there were great mortalities of Eel Grass over extensive areas, particularly the East Coast. Since that time there has been considerable recovery in the east.

2) Phyllospadix scouleri or Surf Grass is found from British Columbia to California. The "sea grasses" include the above four genera and two others (discussed in the families Hydrocharitaceae and Zannichelliaceae. They are stenohaline and probably do not occur where salinities are below 25 ‰ for considerable periods. The remaining genera of this family are primarily fresh water although many have species extending into brackish water.

e Family Potamogetonaceae

Potamogeton includes over forty species and is recognized generally as a difficult group to key out.

f Family Ruppiaceae

Ruppia maritima or Widgeon Grass is found from alkali to fresh water and salt to fresh coastal water.

g Family Zannichelliaceae

1) Zannichellia palustris is found from fresh water and fresh to brackish coastal water.

2) Cymodoceae - Syringodium filiforme (= Cymodocea manatorum) or Manatee Grass is found from Texas to Florida. The leaves are round in cross-section and flowers are common.

3) Halodule wrightii (= Diplanthera) or Shoal Grass is found in North Carolina and from Texas to Florida. Leaves are flat in cross-section and have a three pointed tip.

h Family Najadaceae - includes the one genus Najas, approximately 35 species. Southern Naiad has long been one of the most troublesome submersed aquatic weeds in Florida. It infests irrigation and drainage canals.

i Family Pontederiaceae - includes Eichornia crassipes, waterhyacinth. It is a native of tropical America and was probably introduced in the United States as an ornamental. As an escapee it has become an exceedingly troublesome species by clogging waterways of the Southern States. Its attractive blue-purple flowers and characteristic bulbous leaf stem with rounded leaf blade make it easy to identify. The plant is usually found floating on the surface of ponds and quiet streams and growing on mudbanks. This plant spreads vegetatively by horizontal stem growth and rooting at the nodes to produce new plants that develop into mats covering large areas. The capsulelike fruits contain many seeds that provide for extensive spread of the species in suitable climates.

j Family Acanthaceae - Dianthera (= Justicia) or Water Willow forms large beds at the margins of streams.

k Marsh and shore zones are inhabited by Bulrushes or Sedges (Family Cyperaceae), Rushes (Family Juncaceae), and Grasses (Gramineae). The latter includes wild rice (Zizania)and Cord Grass (Spartina).

l Family Amaranthaceae - includes Alternanthera philoxeroides or Alligator Weed.

m Family Lemnaceae - The Duck weed family includes the smallest known flowering plants, all of which are free floating. Wolffia and Wolffiella are without roots and Lemna and Spirodela both possess them.

n Family Typhaceae - one genus Typha. Cattail or tule.

o Family Iridaceae - Iris pseudacoris is another European plant becoming widely established in North America.

p Family Araceae - Acorus calamus, sweet flag, is an Eurasian plant which has become widely established in North America.

III TYPICAL AQUATIC PLANT COMMUNITIES

A Ponds and lakes not only have a characteristic plant zonation, but over a period of years exhibit a distinct succession of aquatic plant communities.

B Intertidal salt marshes are so distinctive that in one state,regulations preventing the disruption of the ecology of salt marshes, are defined on the basis of the specific plants involved. A typical estuarine shore (from seaward in) might have successive zones dominated by

1 Suaeda (alkali seepweed)

2 Spartina (cord grass)

3 Halimione

4 Puccinellia (alkaligrass) and others

5 Juncus (rush)

C Mangrove vegetation replaces salt marshes in tropical and subtropical regions. Two most widespread genera are·

1 Rhizophora, the red mangrove occupies the outer pioneer zone. Roots are borne on downward curving branches or rhizophores. The resulting tangle reduces tidal currents and promotes deposition of solids.

2 Avicennia, the black mangrove, usually forms a shoreward zone. It depends on aerial roots, which emerge from the ground a short distance from the tree, rather than prop roots.

3 Examples have been given of open shoal areas being transformed into thick swamp forest in some 30 years through this process.

D Sea grass communities, whether composed of Zostera (eel grass) in Northern waters or Thalassia (turtle grass) or Cymodocea (Manatee grass) in the tropics, are recognizable as an entity wherever found. A soft substratum near low water is required, and each has a similar distinct assemblage of molluscs, crustacenas, and other invertebrates.

1 The destruction of this community by bulkheading and filling has far-reaching effects on the stability of such bottom areas.

2 Destruction of these plants by disease, such as the epidemics which decimated the Zostera eel grass community in the thirties created both erosion problems and also brought about far-reaching biotic community changes which only became reestablished with the return of the eel grass.

IV In comparison to other living organisms the aquatic plants or macrophytes have been seriously neglected. Man's increased activities have favored some plants (which we call "weeds") through eutrophication and diminished others (which we call a valuable resource loss) through dredging and filling operations. These events demand reassessment of knowledge about the ecology of these plants and increased contributions toward increasing that information.

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This outline was prepared by R.M. Sinclair, Aquatic Biologist. National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

AN ARTIFICIAL KEY TO SOME COMMON AQUATIC PLANTS

(Freshwater, Estuarine, and Marine)

I Specific identification of aquatic plants often requires microscopic examination of parts which may not always be available to the investigator (for example, fruiting bodies and/or seeds which are seasonal or produced only rarely).

Because of this, some groups are more difficult to identify. Potamogeton is a varied and difficult genus, although Ogden's Key gives help where only vegetative parts are available. Najas and Myriophyllum are often difficult.

In the Hydrocharitaceae, Hydrilla, Egeria, and Elodea (Anacharis is an obsolete name for the latter two) are sometimes confused, and the serious student may need to turn to the specialist in this group (see Blackburn et al for a discussion of this problem.

For a detailed list of references on classification see Sculthorpe (p. 16-20) where these are listed by family. The following key will aid the common plant groups. It is divided as follows.

A) Plants floating on water surface.

B) Plants submersed beneath water surface.

C) Plants erect and emersed, rooted to the substratum and extending upward out of water.

D) Submersed "sea grasses".

Manuals with descriptive comments and figures for specific identification are listed in part under references.

To use the key, select the proper group (A, B, C, D) and read the description in the first couplet. The description that best fits the unknown specimen will indicate either the plant group or genus to which the specimen may belong or an additional couplet, in which case the process is repeated until the description for a particular plant or genus best fits the unidentified specimen. An asterisk * indicates that distribution may include brackish or salt water. Common names used here (with few exceptions) are those of: Subcommittee on Standardization of Common and Botanical Names of Weeds, reprinted from Weeds 14 (4):347-386 (1966).

A PLANTS FLOATING ON WATER SURFACE

1. Plants very small, seldom over a centimeter along any dimension.	2
1. Plants large; usually measuring at least 0.5 decimeter along some dimension.	8
2. Plant body dichotomously 2-lobed, or repeatedly dichotomously branched.	3
2. Plant body not dichotomously branched and if 2-lobed not equally so.	4
3. Divisions of plant body fine and many, plant body usually floating below surface.	Riccia fluitans L.
3. Divisions of plant body coarse, 2-lobed, floating on surface.	Ricciocarpus natans L.
4. Plants floating on surface.	5
4. Plants floating just below water surface; plant body made up of a clump of short filaments.	Wolffiella floridana Sm.
5. Plant body of small overlapping scales.	Azolla
5. Plant body simple or compound, made up of rounded floating leaves.	6

6. Plants simple, extremely minute, appearing as grains on surface of water.	watermeal <u>Wolffia</u>
6. Plants compound, made up of several rounded-oblong disk-like bodies, floating on surface of water.	7
7. Plant body inconspicuously nerved, rootlets 1 per disk.	star duckweed <u>Lemna</u>
7. Plant body conspicuously nerved, rootlets 2 to several per disk.	giant duckweed <u>Spirodela polyrhiza</u> L.
8. Leaves broad and blade-like, sometimes inflated near base.	9
8. Leaves (plant body in Riccia) narrow or finely divided.	12
9. Leaves large and dilated with inflated petioles.	waterhyacinth <u>Eichhornia crassipes</u> Mart.
9. Leaves normally expanded.	11
10. Leaves bearing plantlets around the margin.	floating fern <u>Ceratopteris</u>
10. Leaves not bearing plantlets.	11
11. Floating-leafed plants with leaves attached to the bottom by a bare unbranched stem of varying length.	15
11. Roots usually suspended free in the water, with no connection to lake bottom; capable of drifting.	12
12. Leaves wide to the base, without petioles.	water lettuce <u>Pistia stratiotes</u> L.
12. Leaves mostly differentiated into blades and petioles.	frogbit <u>Limnobium spongia</u> Basc.
13. Plant body repeatedly dichotomously branched.	<u>Riccia fluitans</u> L.
13. Leaves not dichotomously branched.	14
14. Leaves entire not dissected or bearing bladders, and whorled.	<u>Elodea</u>
14. Leaves dissected and bearing bladders.	bladderwort <u>Utricularia</u>
15. Stem attached to middle of leaf.	16
15. Stem attached at the summit of a deep notch in the leaf.	17
16. Leaves oval, not more than 3 inches wide, with supple stem attached to the middle of the leaf.	watershield, <u>Brasenia schreberi</u> Gmelin
16. Circular leaf with a long, fairly rigid stem attached to the middle of the leaf, leaves 6 inches or more wide sometimes supported by the stem above the water level.	american lotus <u>Nelumbo</u>
17. Circular or heart-shaped leaf with the veins radiating from the mid-rib nearly to the margin without forking, floating yellow flowers.	yellow pond lily <u>Nuphar</u>
17. Circular leaf with much-forked veins radiating to the margin, white, or pink floating flowers.	*white water lily <u>Nymphaea</u>

B PLANTS SUBMERSED BENEATH WATER SURFACE

1. Plant body made up of stems bearing whorled, smooth, brittle branches, easily snapped with a slight pressure, plants with a musky odor, no roots, often with a limy encrustation.	*green alga, muskgrass, Chara
1. Plant body not as described.	2
2. Plant attached to substrate by holdfasts or haptera, not rooted.	3
2. Plant not so attached.	4
3. Plant attached to stones by haptera (roots not attached) and tough stems forming a dull-green tangle.	riverweed, Podostemum ceratophyllum
3. Plants single shoots attached to substrate by holdfasts.	red alga Lemanea
4. Without true roots, flowers or vascular bundles.	Division Bryophyta, Mosses and Liverworts 5
4. Usually with true roots and with vascular bundles.	Division Angiospermae. True flowering plants 6
5. Leaves without midrib, arranged in two opposite rows, usually with a part folded under.	Order Jungermanniales, Porella
5. Leaves most often with midrib, usually arranged equally around the stem, not curved under.	Order Musci, Leptodictyum, Fontinalis, and Fissidens
6. Submerged leaves bearing small bladders, leaves irregularly forked.	bladderwort Utricularia
6. Submerged leaves not bladder bearing.	7
7. Submerged leaves compound, made up of narrow segments or leaflets.	8
7. Submerged leaves simple, made up of a single narrow blade.	11
8. Submerged leaves with one central axis, leaves feather-like, branches in whorls about the stem, stems usually very lax.	*water milfoil Myriophyllum
8. Submerged leaves irregularly forking.	9
9. Submerged leaves singly and alternately or irregularly borne; leaves many branches, irregularly forked and appearing as tufts of numerous thread-like projections attached to the center stem.	*water buttercup Ranunculus
9. Submerged leaves borne opposite each other on stem or whorled.	10
10. Leaves stalked, fan-like, extending from opposite sides of the stem, leaflets not toothed.	fanwort Cabomba caraliniana Gr.
10. Stems with whorls of stiff, forked leaves, leaflets with toothed or serrated margins (small barbs) on one side; plant without true roots.	coontail Ceratophyllum

11. Submerged leaves long and ribbon-like, at least 1/10 inch wide.		12
11. Submerged leaves not ribbon-like; often thread-like but if wider than 1/10 inch, less than 1 inch long.		22
12. Leaves scattered along the stem.		13
12. Leaves all borne from one point.		21
13. Leaves with mid-ribs evident when held against bright light, many species with great diversity in leaf forms.	*	pondweed _Potamogeton_ 14
13. Leaves without mid-ribs evident when held against bright light.		water star grass _Heteranthera_
14. Plants with both floating and submerged leaves, the floating leaves with expanded blades and differing from those submerged.		15
14. Plants with all leaves alike and submerged.		18
15. Floating leaves, heart-shaped at the base, 1 to 4 inches long, waxy in appearance.		floating pondweed _Potamogeton natans_ L.
15. Floating leaves rounded at the base.		16
16. Floating leaves with 30 to 50 nerves, submerged leaves about three times as long as broad.		large leaf pondweed _Potamogeton amplifolius_ Tuckerman
16. Floating leaves with less than 30 nerves.		17
17. Upper submerged leaves with long stalks.		American pondweed _Potamogeton nodosus_ Poiret
17. Submerged leaves not as above but with an abrupt awl-shaped tip.		pondweed _Potamogeton angustifolius_ Berchtold
18. Margins of the thin leaves crimped and toothed, the marginal serrations visible to the naked eye.	*	curlyleaf pondweed _Potamogeton crispus_ L.
18. Margins of leaves not visibly toothed.		19
19. Leaves minutely toothed on the margins, visible when magnified, leaves extending stiffly in opposite directions so that whole plant appears flat; only midvein prominent.		Robbins pondweed _Potamogeton robbinsii_ Oakes
19. Not as above		20
20. Stems much flattened and winged, about as wide as the leaves; leaves 1/12 to 1/5 inch wide.		flatstem pondweed _Potamogeton zosteriformis_ Fernald
20. Leaves threadlike, long, rounded, and slender, rarely exceeding 1/10 inch wide, oriented into a lax, diffuse, branched spray. The "bunched" appearance of the threadlike rounded leaves as they float in the water readily distinguished sago pondweed from others of group.	*	sago pondweed _Potamogeton pectinatus_ L.

21. Leaves very long and ribbonlike; when examined with hand lens, showing a central dense zone and a peripheral less dense zone; flowers borne on a long stem that forms a spiral after fertilization. — * Vallisneria

21. Leaf, when examined with hand lens not showing zones as above. — waterplantains, Alismataceae

22. Leaves whorled, 3 - 8 in upper whorls. — Elodea, Egeria, Hydrilla

22. Leaves alternate or opposite. — 23

23. Leaves alternate, leaf-base apparently inflated. — * widgeongrass, Ruppia maritima L.

23. Leaves opposite. — 24

24. All leaves long and narrow. — 25

24. Upper leaves shorter and broader. — waterstarwort, Callitriche

25. Leaves dilated at base. — * naiad, Najas

25. Leaves with narrow bases. — * horned pondweed, Zannichiellia palustris L.

C PLANTS ERECT AND EMERSED, ROOTED TO THE SUBSTRATUM AND EXTENDING UPWARD OUT OF THE WATER.

1. Leaves more than 10 times as long as broad. — 2

1. Leaves less than 10 times as long as broad. — 12

2. Base of stem triangular in cross section, the three angles in some cases so rounded as to make the stem appear almost round. — 3

2. Base of stem not triangular. — 5

3. Three cornered seeds, usually straw colored, enclosed within a loose elongated sac, a low-growing grasslike plant. — sedge, Carex

3. Seeds not enclosed in a loose elongated sac. — 4

4. A single flower or seed-bearing struture on the tip of the stem. — spikerush, Eleocharis

4. Stem with one or more leaves extending beyond the spike or seed-bearing structure. (The hardstem bulrush has long, hard, slender, dark olive-green stems, 1/8 to 3/8 inch at the base, extending 3 to 5 feet above the water surface, the softstream bulrush has soft stems of light green color, 3/10 to 1 inch thick at the base.) — *bulrush, Scirpus

5. Leaf with a collarlike appendage, membranous or composed of hairs at the junction of the leaf blade and that part of the leaf that is wrapped around the stem. — 6

5. Leaf without collarlike appendage mentioned above. — 9

6. Seed or flower-bearing structure composed of scales with fringed margins and over-lapping in a single row.	Cutgrass, Leersia
6. Flower-bearing structure not as above.	7
7. Flowering heads composed of small seeds with long silky hairs, appearing as a silky mass. The root-stocks are stout, making it a difficult plant to pull up. Plants are 6 to 12 feet tall.	common reed, Phragmites
7. Flowering heads not appearing as a silky mass.	8
8. Flowering part of plant much branched, but not as closely packed as in Phragmites. Seeds much larger, about 3/4 inch long. Plants with short roots and easily pulled up.	wildrice, Zizania aquatica L.
8. Spikelets, 6 mm long or more appressed along one side of rachis, grass growing in clumps or solid stands.	*cordgrass, Spartina
9. None of the veins more prominent than others.	10
9. Midvein more prominent than others.	11
10. Flowers borne in closely packed cylindrical spikes, seeds very small. (The common cattail has flat leaves about 1 inch wide, the narrow-leaved cattail has leaves somewhat rounded on the back that are 1/8 to 7/8 inch wide.)	Cattail, Typha
10. Flowers in spherical heads, seeds larger, up to size of corn kernel, leaves shallowly and broadly triangular in cross-section.	burreed, Sparganium
11. Plants aromatic when crushed.	sweetflag Acorus calamus L.
11. Plants not aromatic when crushed, flowers large and showy.	yellow iris Iris pseudacorus L.
12. Leaves arising at intervals along the stem.	13
12. Leaves arising at base of the plant.	14
13. Plants with jointed stems, swollen at the joints, or with creeping rootstocks, stems with alternate, simple leaves.	smartweed, Polygonum
13. Stems prostrate or creeping, branched, and often jointed and rooted at the joints; leaves opposite, spreading plant, often forming floating mats over extensive water areas crowding out other plants, broken off branch fragments root readily, and stems may elongate as much as 200 inches in one season.	alligatorweed, Alternanthera philoxeroides Mart.
14. Fleshy or tuber-bearing rootstocks and rosettes of sheathing basal leaves.	15
14. Not as above, floating plants.	16

15. None of the veins more prominent than others. — *arrowhead Sagittaria

15. Midvein and those descending into the lobes more prominent than others. — arrow arum Peltandra virginia L.

16. Plants floating with fibrous, branched roots and rosettes of stalked leaves, the leaf stalks often inflated and bladder-like. — waterhyacinth Eichhornia crassipes Mart.

16. Plants with floating rosettes of stalked leaves, commonly several rosettes produced on branches of the same plant at the end of flexible, cardlike, sparsely-branched submerged stems; plant thrives at depths of 2 to 5 feet and favors muddy bottoms with high organic content, leaf stalks inflated, but not as conspicuously as in waterhyacinth. — water-chestnut, Trapa natans L.

D SUBMERSED "SEA GRASSES"

1. Leaves round in cross section. — manateegrass, Cymodocea manatorum

2. Leaves flat. — 3

3. Leaves widest at or above the middle. — Halophila

3. Leaves uniform width. — 4

4. Leaf tip blunt. — turtlegrass, Thalassia testudinum

4. Leaf tip pointed. — 5

5. Leaf tip single. — eelgrass, Zostera marina

5. Leaf tip 3-pointed. — shoalgrass, Diplanthera wrightii

ACKNOWLEDGMENT:

This outline was adapted from Keys of Mackenthun and Ingram (1967); Robertson and Eyles (1963); and Humm (1956). The figures are from Eyles and Robertson and Hotchkiss (1967).

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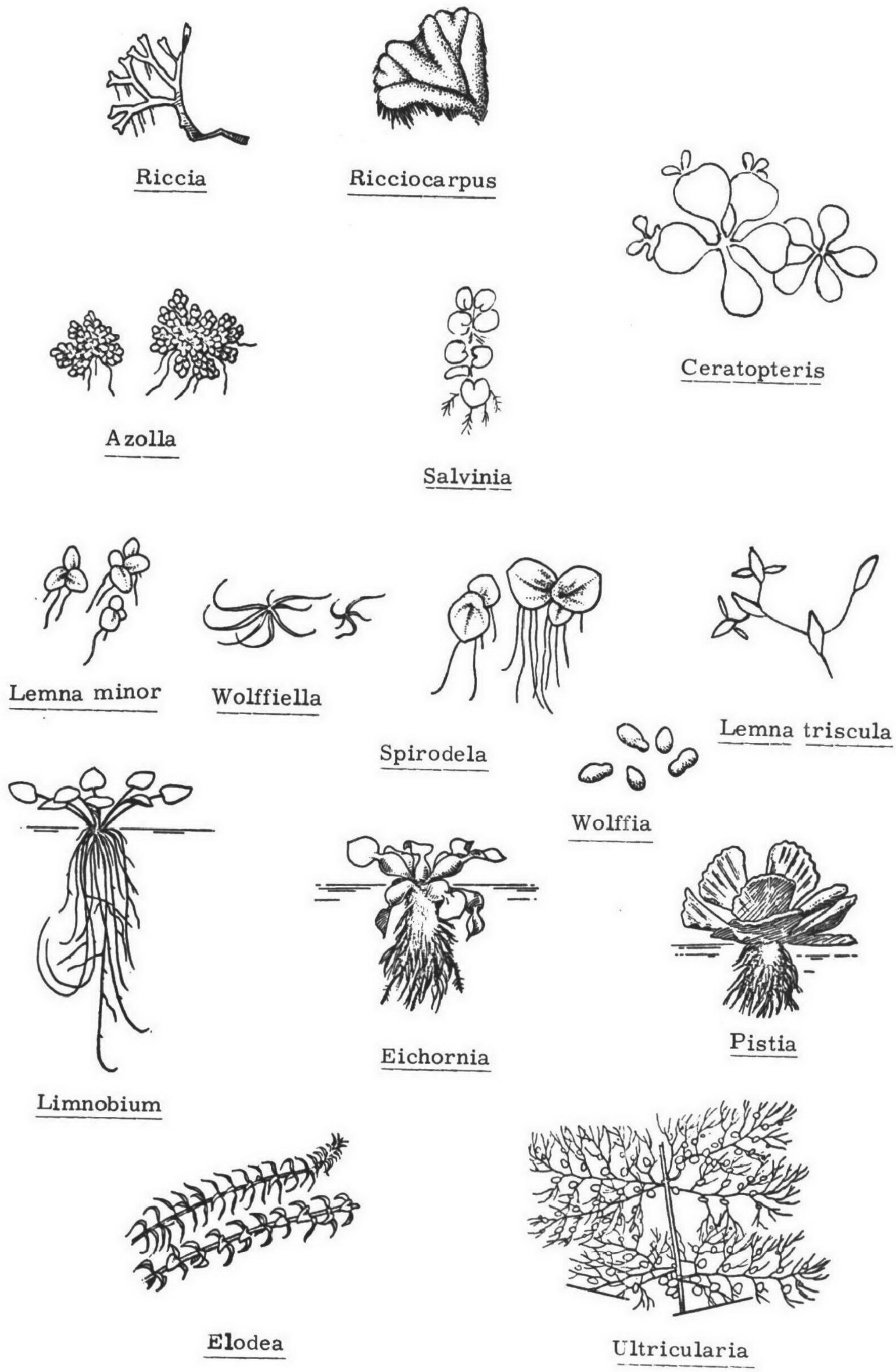
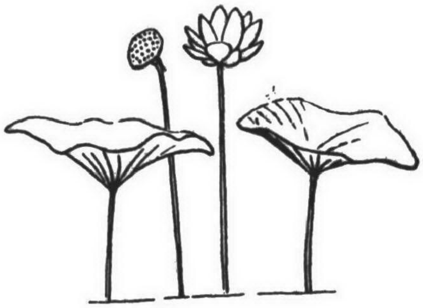
Group A

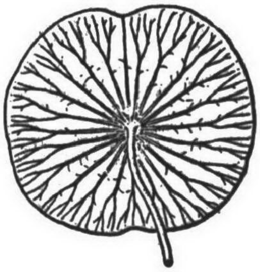
FLOATING PLANTS



Group A

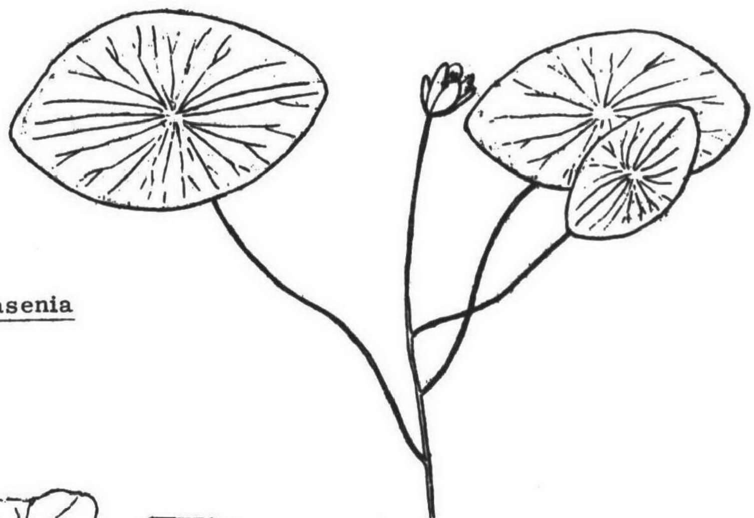
FLOATING PLANTS (Cont.)



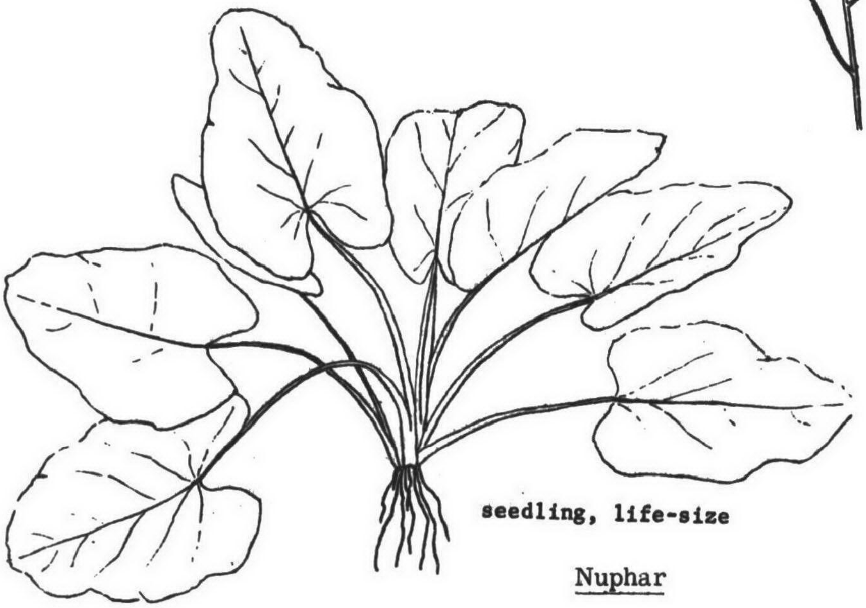


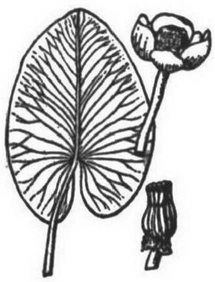
Nelumbo

Nymphaea



Brasenia

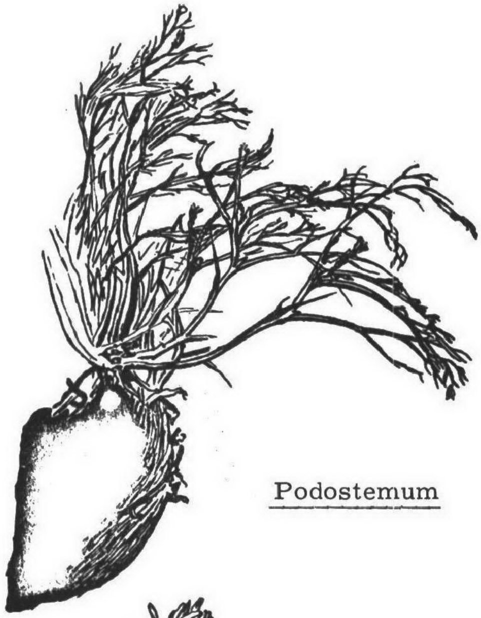




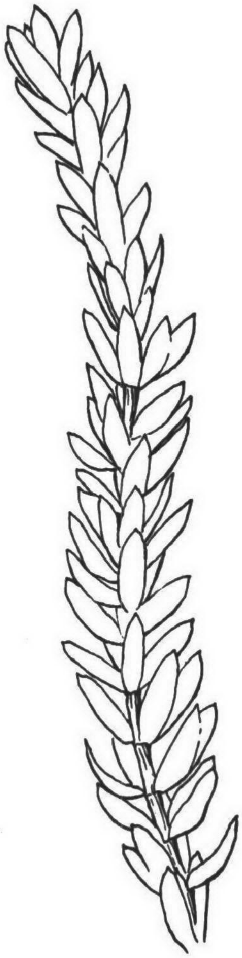
Nuphar

Group B

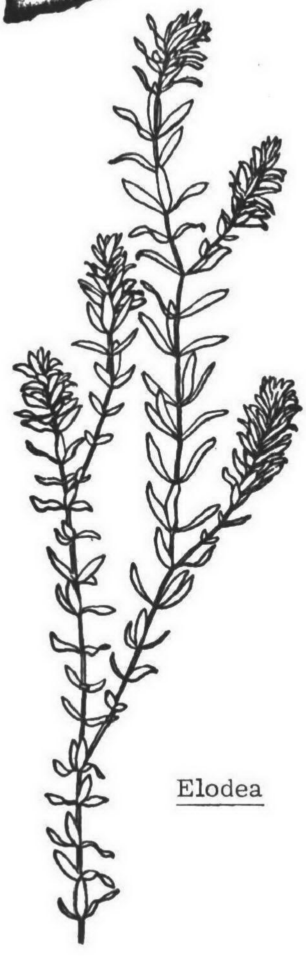
SUBMERSED



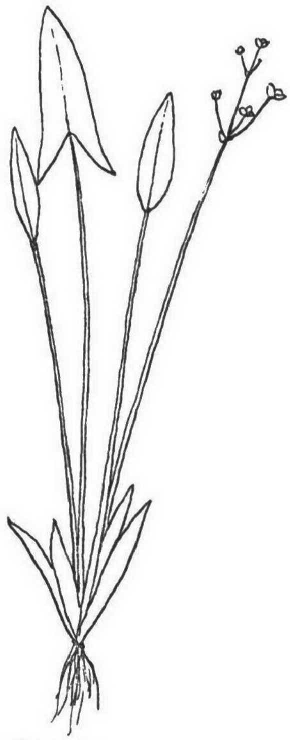
Podostemum



Egeria



Elodea

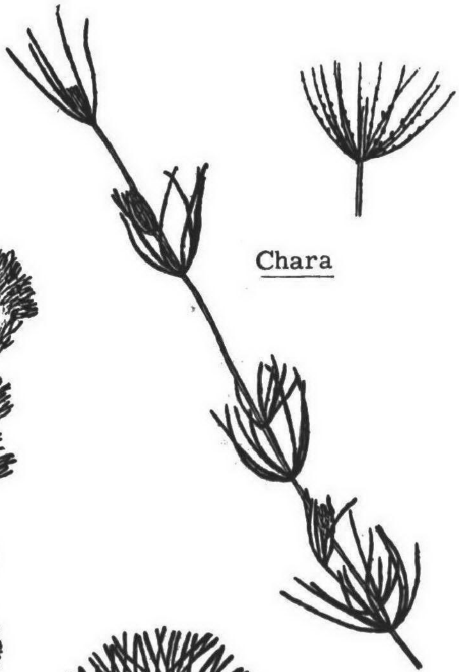


Sagittaria

Group B

SUBMERSED (Cont.)

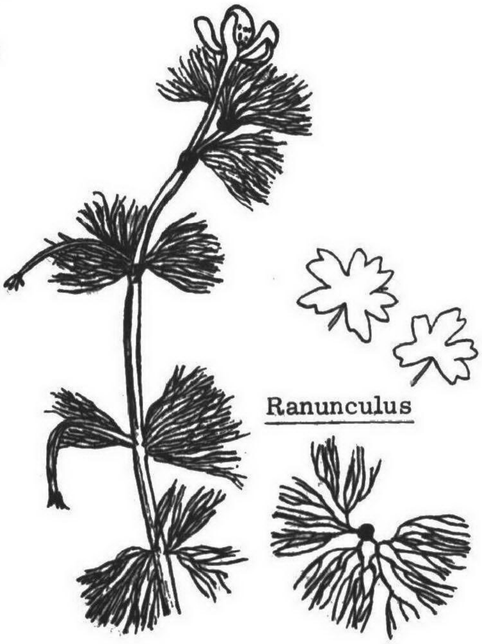
Myriophyllum



Chara

Cabomba

Ceratophyllum



Ranunculus

Group B

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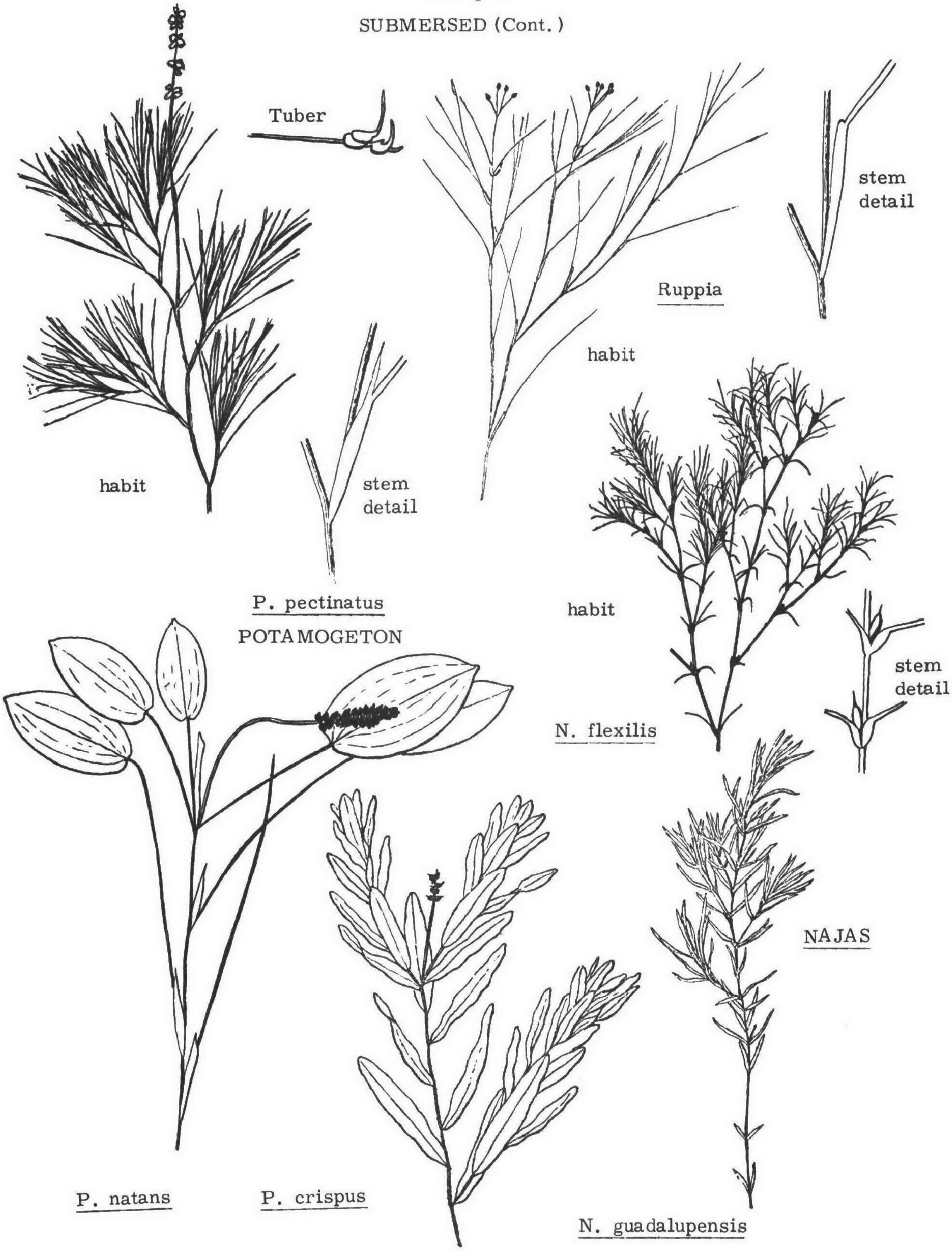
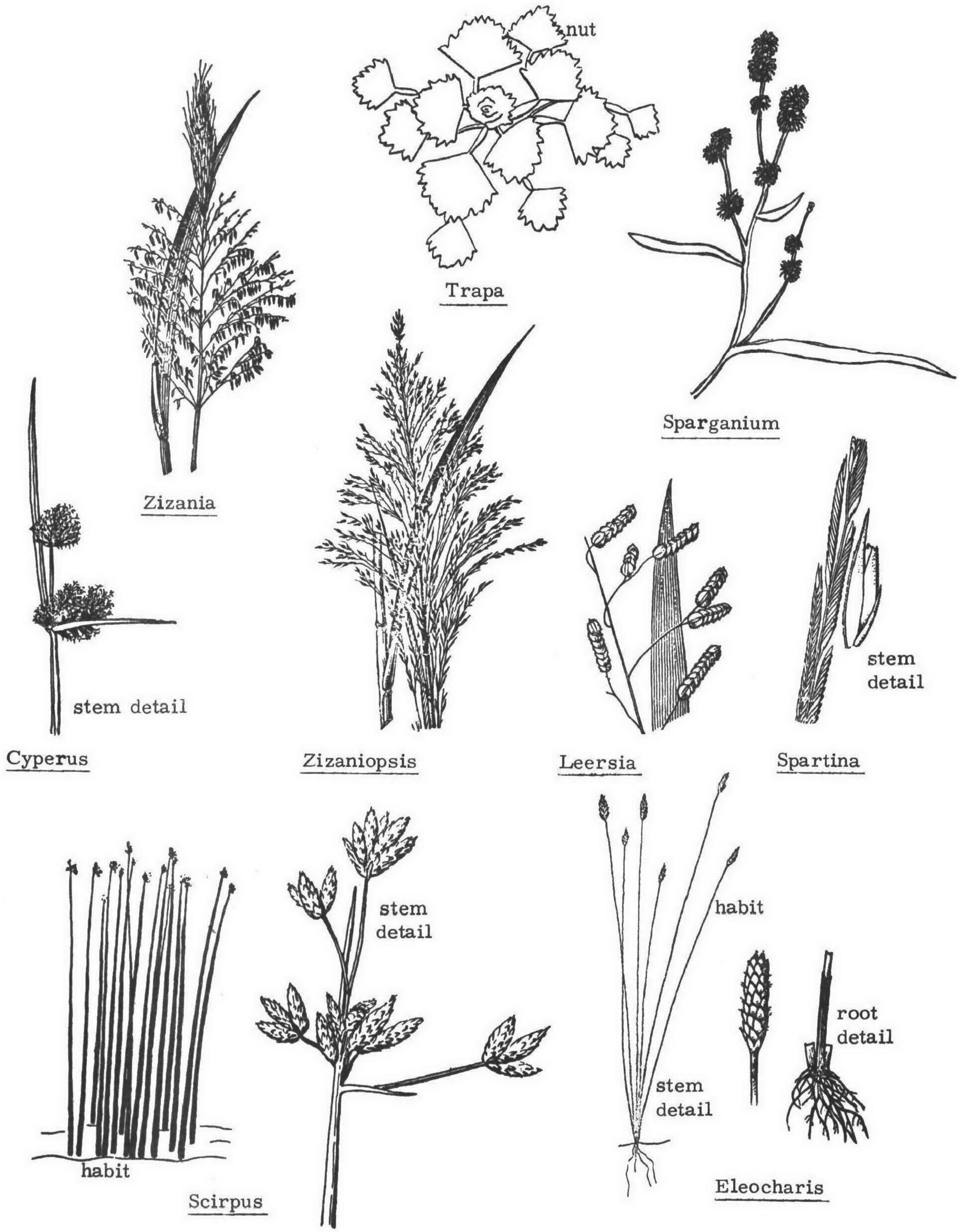
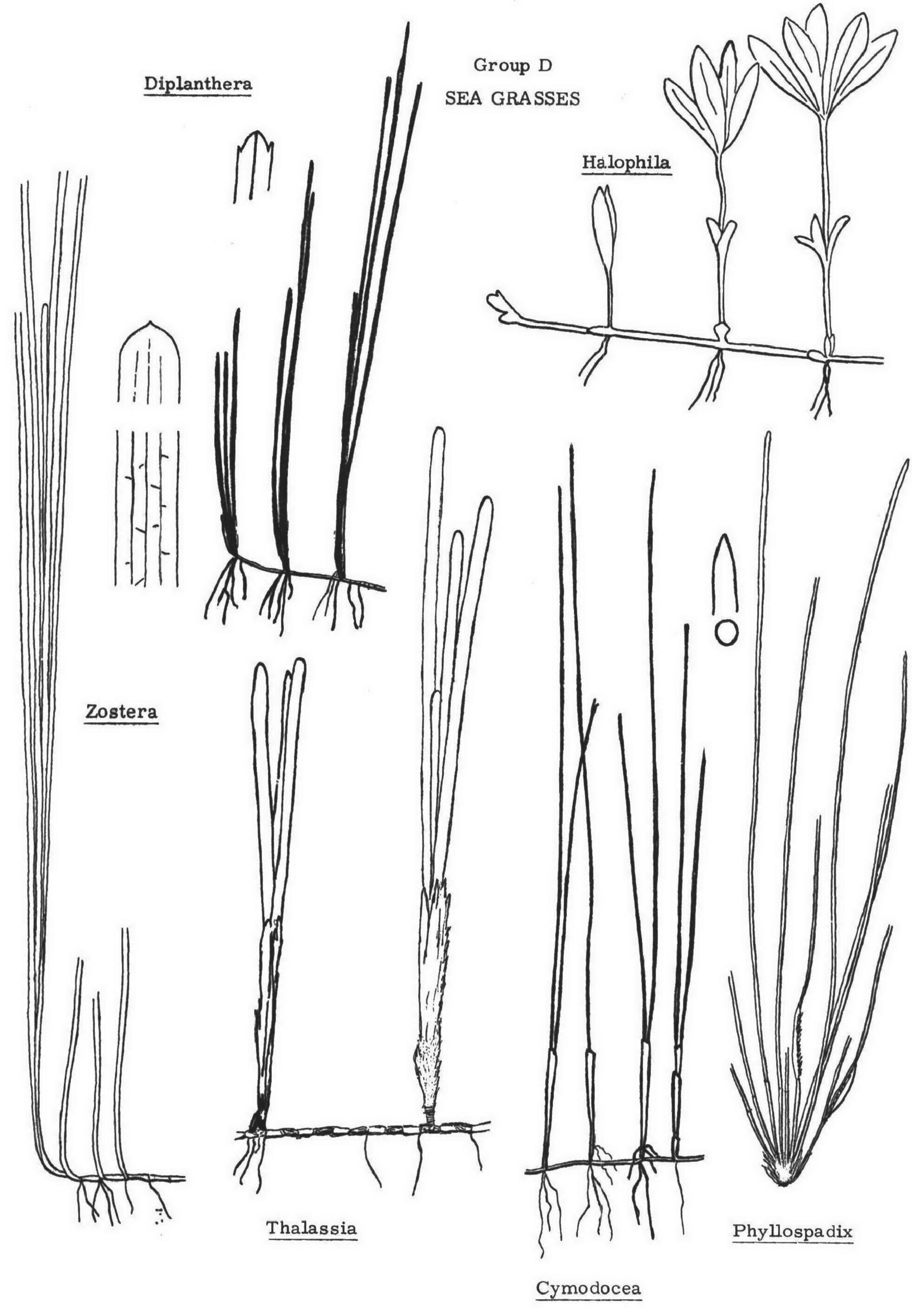


Group C

EMERSED

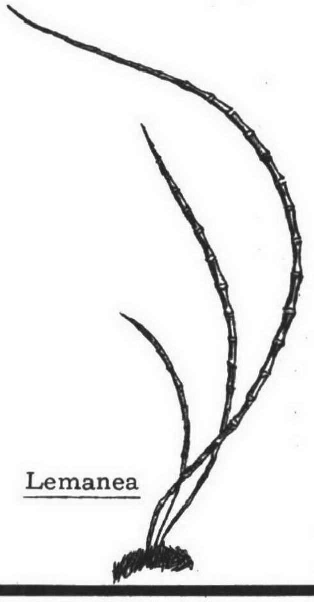




Group D
SEA GRASSES
Diplanthera
Halophila
Zostera
Thalassia
Phyllospadix
Cymodocea

AQUATIC MACROPHYTES - ALGAE AND BRYOPHYTES

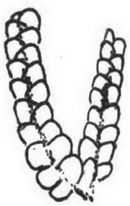
Chara



Lemanea

Nitella

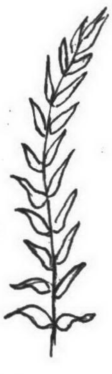
Division ALGAE



Porella

"Liverworts"
Class Hepaticae

Riccia



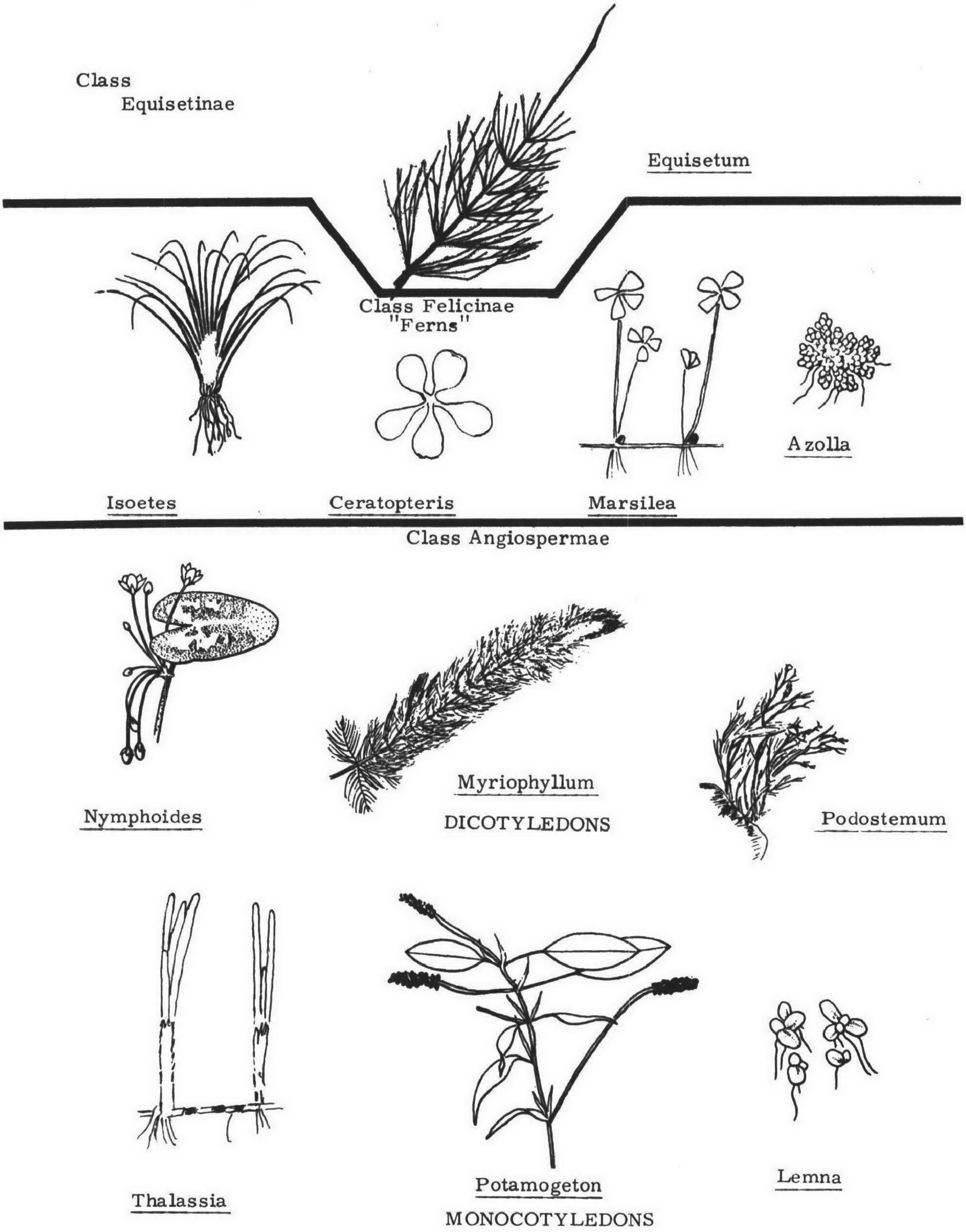
Leptodictyum

"Mosses"
Class Musci

Sphagnum

Division BRYOPHYTA

AQUATIC VASCULAR PLANTS - TRACHEOPHYTA



SECTION C

ANIMALS: CONSUMERS

Animals are consumers; they exist by eating either plants or other animals. The consumer's carbon comes directly or indirectly from the carbon in a plant product; the consumer's utilization of energy comes from the oxidation of some of the plant material back to carbon dioxide and water.

Included in this section are surveys of all major groups of aquatic animals together with a key to selected groups. Ecologically they may be placed in four groups: zooplankton; benthos, nekton; and periphyton, in part.

Contents of Section C

KEY TO SELECTED GROUPS OF FRESHWATER ANIMALS

The following key is intended to provide an introduction to some of the more common freshwater animals Technical language is kept to a minimum

In using this key, start with the first couplet (la, lb), and select the alternative that seems most reasonable. If you selected "la" you have identified the animal as a member of the group, Phylum PROTOZOA If you selected "lb", proceed to the couplet indicated Continue this process until the selected statement is terminated with the name of a group

If you wish more information about the group, consult references (See reference list)

1a The body of the organism comprising a single microscopic independent cell, or many similar and independently functioning cells associated in a colony with little or no difference between the cells, i e , without forming tissues, or body comprised of masses of multinucleate protoplasm Mostly microscopic, single celled animals.

Phylum PROTOZOA

1b The body of the organism comprised of many cells of different kinds, i.e., forming tissues. May be microscopic or macroscopic. 2

2a Body or colony usually forming irregular masses or layers sometimes cylindrical, goblet shaped, vase shaped, or tree like. Size range from barely visible to large. 3

2b Body or colony shows some type of definite symmetry. 4

3a Colony surface rough or bristly in appearance under microscope or hand lens. Grey, green, or brown. Sponges.
Phylum PORIFERA (Fig. 1)

3b Colony surface relatively smooth. General texture of mass gelatinous, transparent. Clumps of minute individual organisms variously distributed. Moss animals, bryozoans.
Phylum BRYOZOA (Fig. 2)

4a Microscopic. Action of two ciliated (fringed) lobes at anterior (front) end in life often gives appearance of wheels. Body often segmented, accordian-like. Free swimming or attached. Rotifers or wheel animalcules.
Phylum TROCHELMINTHES (Rotifera) (Fig. 3)

4b Larger, wormlike, or having strong skeleton or shell. 5

5a Skeleton or shell present. Skeleton may be external or internal. 15

5b Body soft and/or wormlike. Skin may range from soft to parchment-like. 6

6a Three or more pairs of well formed jointed legs present. 19
Phylum ARTHROPODA (Fig. 4)

6b Legs or appendages, if present, limited to pairs of bumps or hooks. Lobes or tenacles, if present, soft and fleshy, not jointed. 7

7a Body strongly depressed or flattened in cross section. 8

7b Body oval, round, or shaped like an inverted "U" in cross section. 10

8a Parasitic inside bodies of higher animals. Extremely long and flat, divided into sections like a Roman girdle. Life history may involve an intermediate host. Tape worms.
Class CESTODA (Fig. 5)

8b Body a single unit. Mouth and digestive system present, but no anus. 9

9a External or internal parasite of higher animals. Sucking discs present for attachment. Life history may involve two or more intermediate hosts or stages. Flukes.
Class TREMATODA

9b Free living. Entire body covered with locomotive cilia. Eye areas in head often appear "crossed". Free living flatworms.
Class TURBELLARIA (Fig. 6)

10a Long, slender, with snake-like motion in life. Covered with glistening cuticle. Parasitic or free-living. Microscopic to six feet in length. Round worms.
Phylum NEMATHELMINTHES (Fig. 7)

10b Divided into sections or segments 11

10c Unsegmented, head blunt, one or two retractile tentacles. Flat pointed, tail. 18

11a Head a more or less well-formed, hard, capsule with jaws, eyes, and antennae.
Class INSECTA order DIPTERA (Figs. 8A, 8C)

11b Head structure soft, except jaws (if present). Fig. 8E.) 12

12a Head conical or rounded, lateral appendages not conspicuous or numerous. 13

12b Head somewhat broad and blunt. Retractile jaws usually present. Soft fleshy lobes or tentacles, often somewhat flattened, may be present in the head region. Tail usually narrow. Lateral lobes or fleshy appendages on each segment unless there is a large sucker disc at rear end. 14
Phylum ANNELIDA (Fig. 9)

13a Minute dark colored retractile jaws present, body tapering somewhat at both ends, pairs or rings of bumps or "legs" often present, even near tail.
Class INSECTA Order DIPTERA (Fig. 8)

13b No jaws, sides of body generally parallel except at ends. Thickened area or ring usually present if not all the way back on body. Clumps of minute bristles on most segments. Earthworms, sludge-worms. 14
Order OLIGOCHAETA

14a Segments with bristles and/or fleshy lobes or other extensions. Tube builders, borers, or burrowers. Often reddish or greenish in color. Brackish or fresh water. Nereid worms.
Order POLYCHAETA (Fig. 9A)

14b Sucker disc at each end, the large one posterior. External blood-sucking parasites on higher animals, often found unattached to host. Leaches.
Class HIRUDINEA (Fig. 9B)

15a Skeleton internal, of true bone. (Vertebrates) 40

15b Body covered with an external skeleton or shell. 16
(Figs. 10, 13, 17, 18, 24, 25, 28)

16a External skeleton jointed, shell covers legs and other appendages, often leathery in nature. 19
Phylum ARTHROPODA

16b External shell entire, not jointed, unless composed of two clam-like halves. 17
(Figs. 10, 11, 12)

17a Half inch or less in length. Two leathery, clam-like shells. Soft parts inside include delicate, jointed appendages. Phyllopods or branchiopods.
Class CRUSTACEA, Subclasses BRANCHIOPODA (Fig. 12) and OSTRACODA (Fig. 11)

17b Soft parts covered with thin skin, mucous produced, no jointed legs. 18
Phylum MOLLUSCA

18a Shell single, may be a spiral cone. Snails.
Class GASTROPODA (Fig. 13)

18b Shell double, two halves, hinged at one point. Mussels, clams.
Class BIVALVIA (Fig. 10)

19a Three pairs of regular walking legs, or their rudiments. Wings present in all adults and rudiments in some larvae. 29
Class INSECTA (Figs. 22, 24D, 25, 26, 28, 29)

19b More than three pairs of legs apparently present. 20

20a Body elongated, head broad and flat

with strong jaws. Appendages following first three pairs of legs are rounded tapering filaments. Up to 3 inches long. Dobson fly and fish fly larvae.
Class INSECTA Order MEGALOPTERA (Fig. 14)

20b Four or more pairs of legs. 21

21a Four pairs of legs. Body rounded, bulbous, head minute. Often brown or red. Water mites.

Phylum ARTHROPODA, Class ARACHNIDA, Order ACARI (Fig. 15)

21b Five or more pairs of walking or swimming legs; gills, two pairs of antennae. Crustaceans. 22
Phylum ARTHROPODA, Class CRUSTACEA

22a Ten or more pairs of flattened, leaflike swimming and respiratory appendages. Many species swim constantly in life, some swim upside down. Fairy shrimps, phyllopods, or branchipods.
Subclass BRANCHIOPODA (Fig. 16)

22b Less than ten pairs of swimming or respiratory appendages. 23

23a Body and legs inclosed in bivalved (2 halves) shell which may or may not completely hide them. 24

23b Body and legs not enclosed in bivalve shell. May be large or minute. 26
(Figs. 17, 18, 19)

24a One pair of branched antennae enlarged for locomotion, extend outside of shell (carapace). Single eye usually visible. "Water fleas"
Subclass CLADOCERA (Fig. 12)

24b Locomotion accomplished by body legs, not by antennae. 25

25a Appendages leaflike, flattened, more than ten pairs.
Subclass BRANCHIOPODA (See 22 a)

25b Animal less than 3 mm, in length. Appendages more or less slender and jointed, often used for walking. Shells opaque. Ostracods.
(Fig. 11) Subclass OSTRACODA

26a Body a series of six or more similar segments, differing mainly in size. 27

26b Front part of body enlarged into a somewhat separate body unit (cephalothorax) often covered with a single piece of shell (carapace). Back part (abdomen) may be relatively small, even folded underneath front part. (Fig. 19b) 28

27a Body compressed laterally, i.e., organism is tall and thin. Scuds. amphipods.
Subclass AMPHIPODA (Fig. 17)

27b Body compressed dorsoventrally, i.e., organism low and broad. Flat gills contained in chamber beneath tail. Sowbugs.
Subclass ISOPODA (Fig. 18)

28a Abdomen extending straight out behind, ending in two small projections. One or two large masses of eggs are often attached to female. Locomotion by means of two enlarged, unbranched antennae, the only large appendages on the body. Copepods.
Subclass COPEPODA (Fig. 19)

28b Abdomen extending out behind ending in an expanded "flipper" or swimming paddle. Crayfish or craw fish. Eyes on movable stalks. Size range usually from one to six inches.
Subclass DECAPODA

29a Two pairs of functional wings, one pair may be more or less hardened as protection for the other pair. Adult insects which normally live on or in the water. (Figs. 25, 28) 39

29b No functional wings, though pads in which wings are developing may be visible. Some may resemble adult insects very closely, others may differ extremely from adults. 30

30a External pads or cases in which wings develop clearly visible.(Figs. 24, 26, 27) 35

30b More or less wormlike, or at least no external evidence of wing development. 31

31a No jointed legs present. Other structures such as hooks, sucker discs, breathing tubes may be present. Larvae of flies, midges, etc.
Order DIPTERA (Fig. 8)

31b Three pairs of jointed thoracic legs, head capsule well formed. 32

32a Minute (2-4mm) living on the water surface film. Tail a strong organ that can be hooked into a "catch" beneath the thorax. When released animal jumps into the air. No wings are ever grown. Adult springtails.
Order COLLEMBOLA (Fig. 20)

32b Larger (usually over 5 mm) wormlike, living beneath the surface. 33

33a Live in cases or webs in water. Cases or webs have a silk foundation to which tiny sticks, stones, and/or bits of debris are attached. Abdominal segments often with minute gill filaments. Generally cylindric in shape. Caddisfly larvae.
Order TRICHOPTERA (Fig. 21)

33b Free living, build no cases. 34

34a Somewhat flattened in cross section and massive in appearance. Each abdominal segment with rather stout, tapering, lateral filaments about as long as body is wide. Alderflies, fishflies, and dobsonflies.
Order MEGALOPTERA (Fig. 22, 14)

34b Generally rounded in cross section. Lateral filaments if present tend to be long and thin. A few forms extremely flattened, like a suction cup. Beetle larvae.
Order COLEOPTERA (Fig. 23)

35a Two or three filaments or other structures extending out from end of abdomen. 37

35b Abdomen ending abruptly, unless terminal segment itself is extended as single structure.(Figs. 24A, 24C) 36

36a Mouth parts adopted for chewing. Front of face covered by extensible folded mouthparts often called a "mask". Head broad, eyes widely spaced. Nymphs of dragonflies or darning needles.
Order ODONATA (Figs.24A, 24C, 24E)

36b Mouthparts for piercing and sucking. Legs often adapted for water locomotion. Body forms various. Water bugs, water scorpions, water boatmen, backswimmers, electric light bugs, water striders, water measurers, etc.
Order HEMIPTERA (Fig. 25)

37a Tail extensions (caudal filaments) two. Stonefly larvae.
Order PLECOPTERA (Fig. 26)

37b Tail extensions three, at times greatly reduced in size. 38

38a Tail extensions long and slender. Rows of hairs may give extensions a feather-like appearance. Mayfly larvae.
Order EPHEMEROPTERA (Fig. 27)

38b Tail extensions flat, elongated plates. Head broad with widely spaced eyes, abdomen relatively long and slender. Damselfly nymths.
Order ODONATA (Fig. 24D)

39a External wings or wing covers form a hard protective dome over the inner wings folded beneath, and over the abdomen Beetles.
Order COLEOPTERA (Fig 28)

39b External wings leathery at base, Membranaceous at tip Wings sometimes very short. Mouthparts for piercing and sucking Body form various True bugs.
Order HEMIPTERA (Fig 25)

40a Appendage present in pairs (fins, legs, wings) 42

40b No paired appendages. Mouth a round suction disc 41

41a Body long and slender Several holes along side of head Lampreys.
Sub Phylum VERTEBRATA, Class CYCLOSTOMATA

41b Body plump, oval. Tail extending out abruptly. Larvae of frogs and toads Legs appear one at a time during metamorphosis to adult form. Tadpoles.
Class AMPHIBIA

42a Paired appendages are legs 43

42b Paired appendages are fins, gills covered by a flap (operculum) True fishes
Class PISCES

43a Digits with claws, nails, or hoofs 44

43b Skin naked. No claws or digits Frogs, toads, and salamanders
Class AMPHIBIA

44a Warm blooded 45

44b Cold blooded Body covered with horny scales or plates
Class REPTILIA

45a Body covered with feathers Birds
Class AVES

45b Body covered with hair Mammals
Class MAMMALIA

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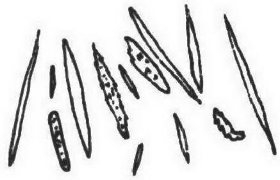
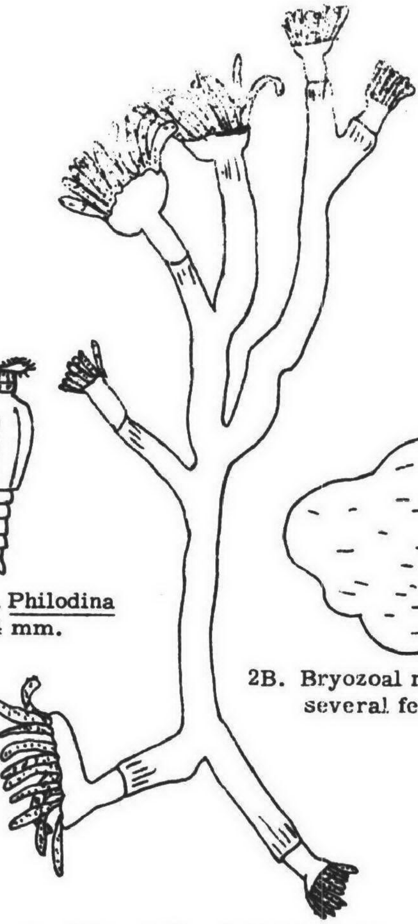
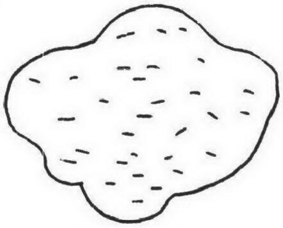


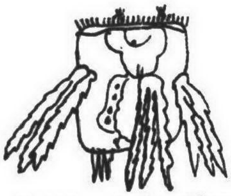
1. Spongilla spicules
Up to .2 mm. long.



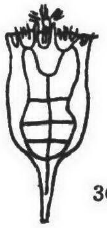
2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.



2B. Bryozoal mass. Up to several feet diam.



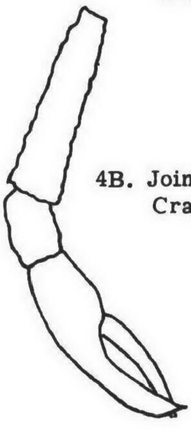
3A. Rotifer, Polyarthra
Up to .3 mm.



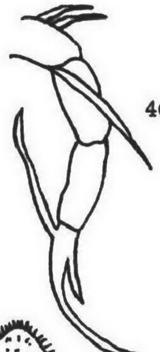
3B. Rotifer, Keratella
Up to .3 mm.

3C. Rotifer, Philodina
Up to .4 mm.

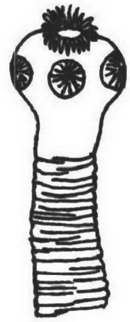
4A. Jointed leg
Caddisfly



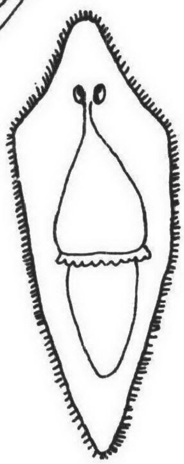
4B. Jointed leg
Crayfish



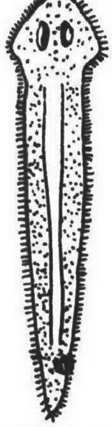
4C. Jointed leg
Ostracod



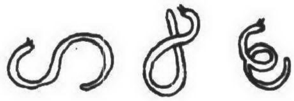
5. Tapeworm head, Taenia. Up to 25 yds. long



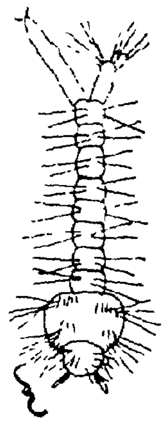
'aria, Mesostoma
) to 1 cm.



6B. Turbellaria, Dugesia
Up to 1.6 cm.

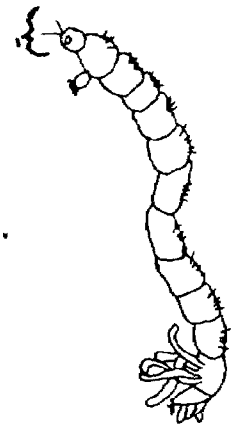


7. Nematodes. Free living forms commonly up to 1 mm., occasionally more.

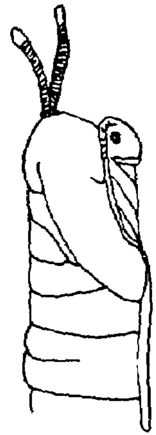


8A. Diptera, Mosquito larvae Up to 15 mm. long.

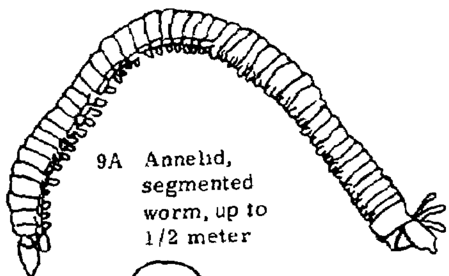
8B Diptera, Mosquito pupa Up to 5 mm.



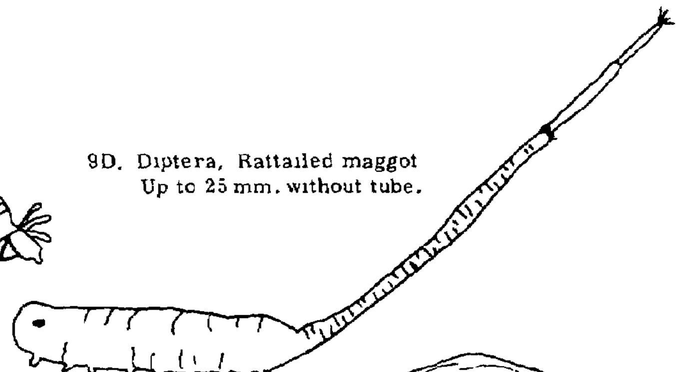
8C. Diptera, chironomid larvae. Up to 2 cm.



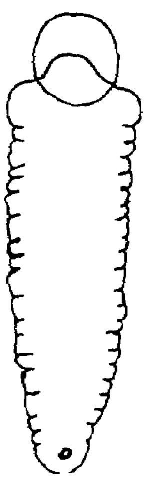
8E Diptera, crane fly pupa Up to 2 5 cm



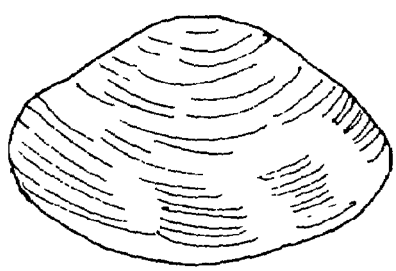
9A Annelid, segmented worm, up to 1/2 meter



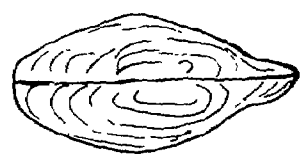
9D. Diptera, Rattailed maggot Up to 25 mm. without tube.



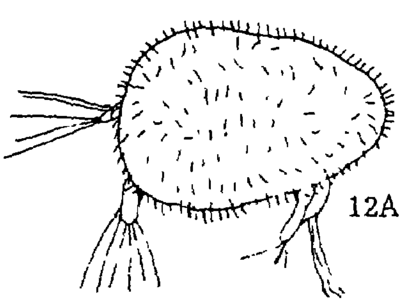
9B. Annelid, leech up to 20 cm.



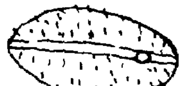
10A. Pelecyopod, Alasmidonta Side view, up to 18 cm. long.



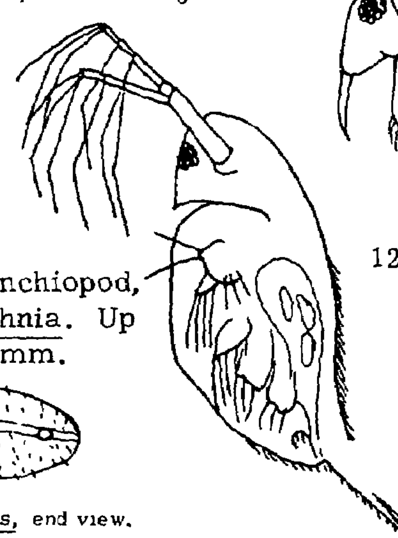
10B. Alasmidonta, end view.



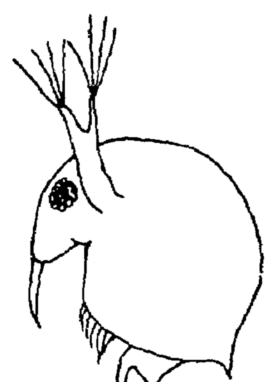
11A. Ostracod, Cypericus Side view, up to 7 mm.



11B. Cypericus, end view.

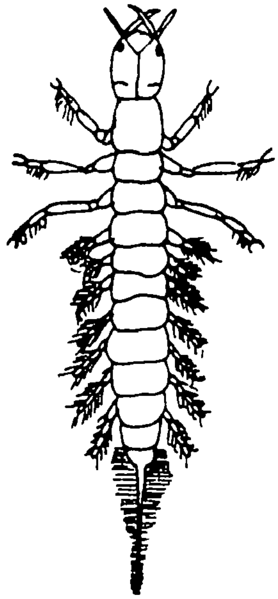


12A. Branchiopod, Daphnia. Up to 4mm.

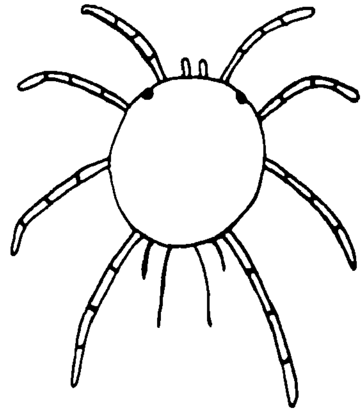


12B. Branchiopod, Bosmina. Up to 2mm.

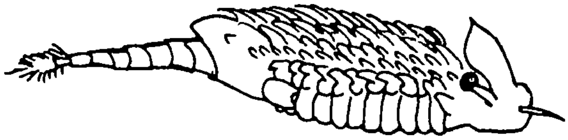
13. Gastropod, Campeloma
Up to 3 inches.



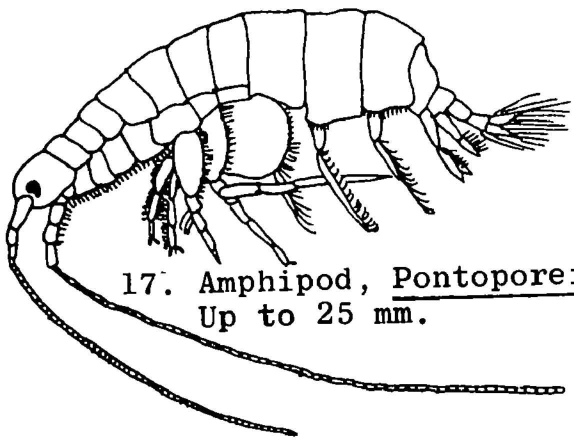
14. Megaloptera, Sialis
Alderfly larvae
Up to 25 mm.



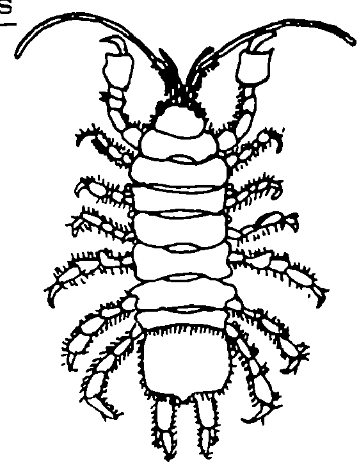
15. Water mite,
up to 3 mm.



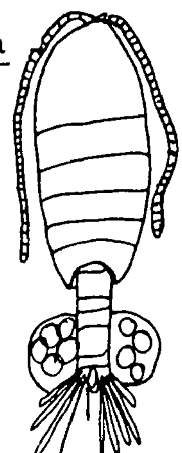
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



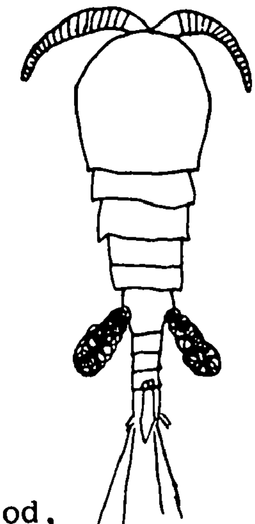
17. Amphipod, Pontoporeia
Up to 25 mm.



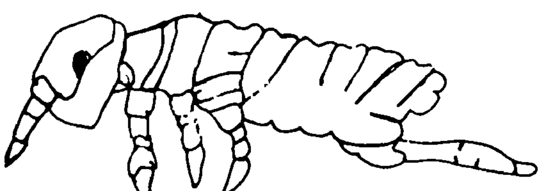
18. Isopod, Asellus
Up to 25 mm.



19A. Calanoid copepod,
Female
Up to 3 mm.

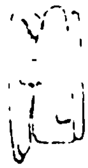


19B. Cyclopoid copepod
Female
Up to 25 mm.



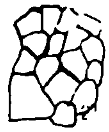
20. Collembola, Podura
Up to 2 mm. long

21A.

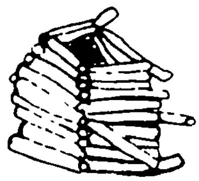


21B.

21C.

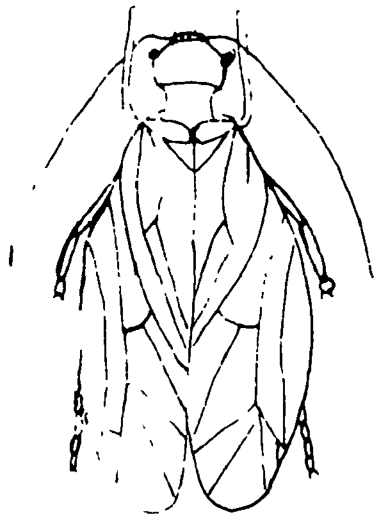


21D.

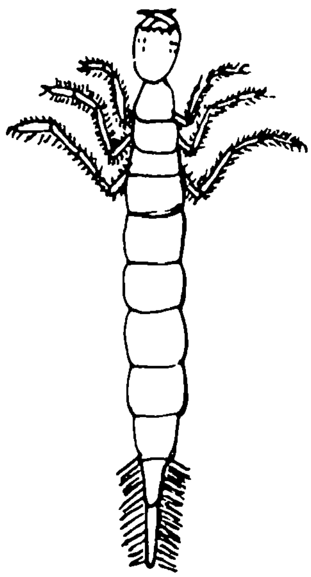


21E.

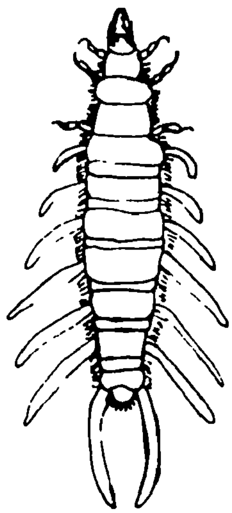
21. Trichoptera, larval cases, mostly 1-2 cm.



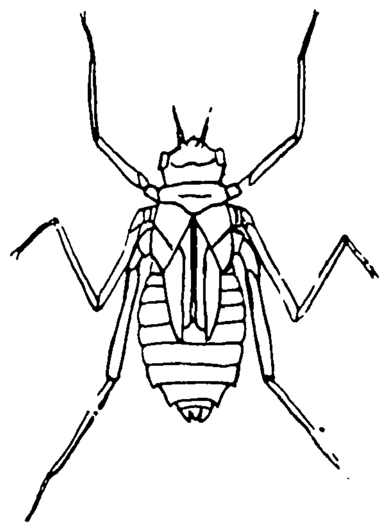
22. Megaloptera, alderfly Up to 2 cm.



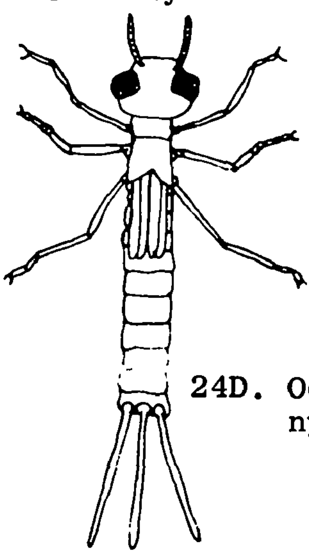
23A. Beetle larvae, Dytisidae, Usually about 2 cm.

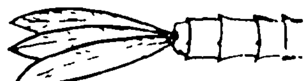


23B. Beetle larvae, Hydrophilidae Usually about 1 cm.



24A. Odonata, dragonfly nymph up to 3 or 4 cm

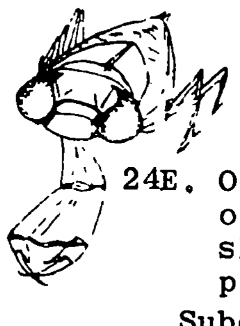




24B. Odonata, tail of damselfly nymph (side view)

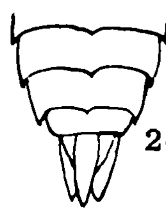
Suborder Zygoptera (24B, D)

24D. Odonata, damselfly nymph (top view)

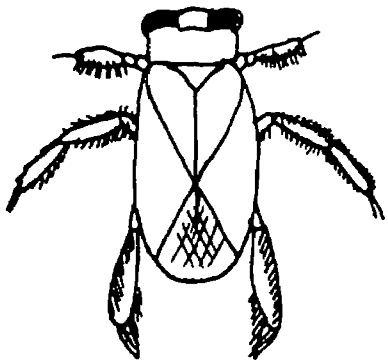


24E. Odonata, front view of dragonfly nymph showing "mask" partially extended

Suborder Anisoptera (24A, E, C)

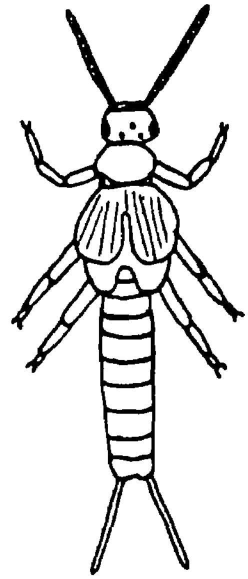


24C. Odonata, tail of dragonfly nymph (top view)

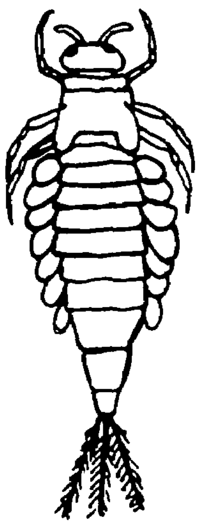


25A. Hemiptera,
Water Boatman
About 1 cm.

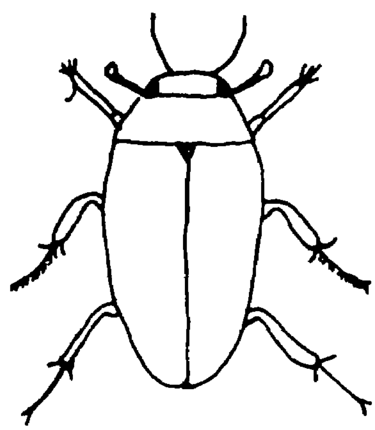
25B. Hemiptera,
Water Scorpion
About 4 cm.



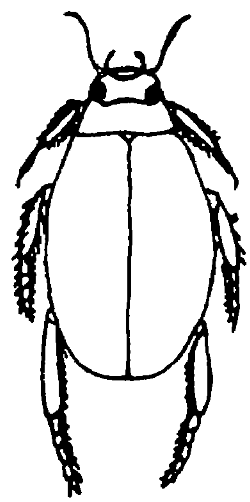
26. Plecoptera,
Stonefly nymph
Up to 5cm.



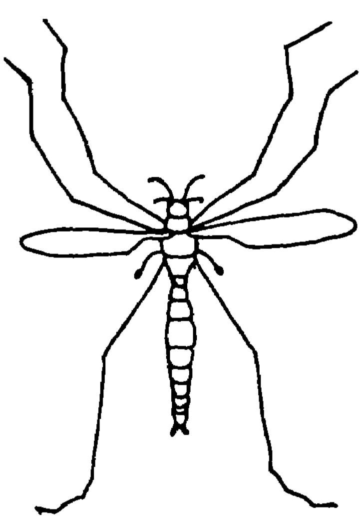
27. Ephemeroptera,
Mayfly nymph
Up to 3cm.



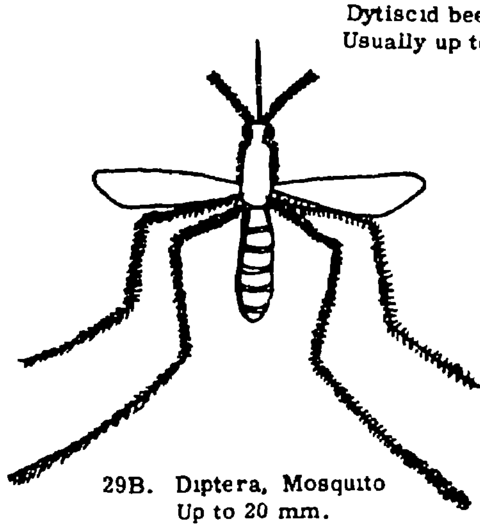
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



28B. Coleoptera,
Dytiscid beetle
Usually up to 4 cm.



29A. Diptera, Crane
fly. Up to $2\frac{1}{2}$ cm.



29B. Diptera, Mosquito
Up to 20 mm.

BIOTA OF WASTEWATER TREATMENT PLANTS (MICROSCOPIC INVERTEBRATES)

I GENERAL CONSIDERATIONS

A Community rather than individual as a unit for study of the process, quantitative relationship among different populations - "population dynamics".

B Sequential transformation of organic matter through the microbial life - a transference of materials and energy between microbial populations led to the development of functional synecology or productive ecology

C Microbes considered here include bacteria, protozoa, and microscopic metazoa, algae and fungi are important groups included elsewhere in more detail.

D All microbial groups originate from a) the waste itself, b) washing waters, c) soil, d) dust from air, and e) incidental sources, only those members that can survive and establish themselves in the community are important, some are transient.

E Some variations in composition of the microbial community in domestic sewage treatment due to climatic and other ecological factors, industrial wastes with specific waste matter may call for development of more restricted microbial community for degradation.

F Most active microbial groups are True bacteria, filamentous bacteria, fungi, protozoa, nematodes, rotifers, oligochaetes, and water-mites.

II BACTERIA

A No ideal method for studying distribution and ecology of bacteria in waste-treatment. Total bacterial counts made on nutrient agar or gelatine reflect only a portion of the bacterial flora present.

B Pseudomonads are probably the most versatile in their ability to attack a great variety of organic compounds, including petroleum products, phenolics, cyanides. Others, such as Achromobacter, Alcaligenes, Chromobacterium, Flavobacterium, Aerobacter, and Micrococcus, are also important genera Actinomyces are prominent in wastes rich in cellulose and Bacillus organisms are starch attackers. Sulfur and iron bacteria are predominant in wastes rich in respective compounds

C Actinomyces, Bacillus spp , Aerobacter spp., and nitrogen-fixation bacteria are primarily soil dwellers and are almost always present in any type of wastes in small numbers.

D Parasitic and pathogenic bacteria, if present, are transient.

E In extended aeration process with high dissolved oxygen, predominant species are limited to pseudomonads, Zoogloea ramigera, and Sphaerotilus

III PROTOZOA

A Classification

1 Single-cell animals in the phylum Protozoa in the animal kingdom.

or

2 A separate kingdom, Protista, to include protozoa, algae, fungi, and bacteria.

a Mastigophora (flagellates) - only the subclass Zoomastigina (non-pigmented) included, four orders:

1) Rhizomastigina - amoeba-flagellates with 1 or more flagella, examples Mastigamoeba Actinomonas, Rhizomastix

2) Protomonadina - with 1 or 2 flagella, comprising most of the free-living forms, examples: Peranema, Bodo, Monas, Pleuromonas

3) Polymastigina - with 3-8 flagella, mostly parasitic in gut of man and animals

4) Hypermastigina - with numerous flagella, all parasitic in insect intestine

b Ciliophora or Infusoria (ciliates) - largest class of protozoa, no pigmented members, most important group of protozoa in waste treatment, 2 subclasses.

1) Ciliata - cilia present during the the entire trophic life, comprising most of the common ciliates, examples: Paramecium, Colpidium, Colpoda, Euplotes, Stylonychia, Vorticella, Opercularia, Epistylis, Carchesium

2) Suctoria - cilia present while young and tentacles during trophic life

c Sarcodina (amoebae) - pseudopodia (false feet) for locomotion and food-capturing, cell without cell-wall, some with test or shell, 2 subclasses

1) Rhizopoda - pseudopodia without axial filaments, 5 orders:

a) Proteomyxa - with radiating pseudopodia, no test or shell

b) Mycetazoa (slime-molds) forming plasmodium, resembling fungi in sporangium formation.

c) Amoebina - true amoeba, pseudopodia in the form of lobopodia, no test or shell, cyst formation frequent, a few capable of flagellate transformation, examples: Naegleria, Amoeba, Hartmannella, Endamoeba

d) Testacea - amoeba with single test or shell, examples Arcella, Difflugia

e) Foraminifera - large amoeba with calcareous shell, all marine forms

2) Actinopoda - with spinous pseudopodia, 2 orders

a) Heliozoa - without central capsule, usually spherical in form with many radiating axopodia, examples Actinosphaerium, Actinophrys

b) Radiolaria - pelagic in various oceans

d Sporozoa - no organ of locomotion, all parasitic (Plasmodium, Coccidia)

B General Morphology

1 Zoomastigina:

With the exception of Rhizomastigina which is amoeboid, the body has definite shape (oval, leaf-like, pear-like, etc.); most free-living forms with 1-2 flagella, some with 3 or more flagella, few forming colonies, cytostome present in many for feeding on bacteria, relatively small size (15-40 μ)

2 Ciliophora

Most highly developed protozoa, with few exceptions, a macro- and a micronucleus, adoral zone, mouth, oral groove, usually present in swimming and crawling forms, stalked form with conspicuous ciliation of a disc-like anterior region and little or no body cilia, cyst formed in most species

3 Sarcodina

Cytoplasmic membrane but not cell-wall, cytoplasm with distinct ectoplasm and endoplasm in many common spp , nucleus with large nucleolus in most of the free-living forms, some with the body enclosed in a test or shell and moving by protruding pseudopodia outside of the enclosure through an opening, few capable of temporary transformation into flagellate, fresh-water actinopods usually spherical with many radiating axopodia, some Testacea spp. containing symbiotic algae - mistaken for pigmented amoebae, cysts with single or double wall and 1-2 nuclei, parasitic amoebae forming cysts with 4 or more nuclei

C General Physiology

1 Zoomastigina

Free-living forms normally holozoic, food supply mostly bacteria, relatively aerobic, therefore, among the first to disappear in anaerobic conditions, reproduction by simple fission and occasionally by budding.

2 Ciliophora

Holozoic, true ciliates concentrating food particles, i.e , bacteria, by ciliary movement around the mouth-part, suctoria sucking through tentacles, bacteria, small algae and protozoa constituting main food under normal conditions, not as aerobic as flagellates - a few surviving under highly anaerobic conditions, such as Metopus, reproducing by simple fission, conjugation, or encystation.

3 Sarcodina

Mostly holozoic, feeding through engulfing by pseudopodia, food supply of small amoebae mostly bacteria, large amoebae engulfing larger organisms, shelled amoebae, i.e , Arcella, feeding on a variety of organisms or saprozoic, reproduction by simple fission and encystation.

IV NEMATODA

A Classification

1 All in the phylum Nemata (nonsegmented round worms), 2 subphyla:

Secernentea (phasmids) 6 orders Tylenchida (spear in mouth), Rhabditida (rhabditoid eosophagus), Strongylida (parasitic), Ascaridida (parasitic), Spirurida (parasitic), and Camallanida (parasitic), with the exception of tylenchids, all with papillae on male tail

Adenophora (aphasmids) 5 orders· Dorylaimida (spear in mouth), Chromodorida, Monhysterida, Enoplida, and Diocytophymatida, no papillae on male tail, no excretary canal

2 Nematodes encountered in polluted water and in sewage treatment mostly belonging to order Rhabditida and few in orders Dorylaimida and Tylenchida, those in Rhabditida being bacteria-feeders and those in the latter two, feeding on algae and other zoomicrobes, examples of rhabditids Rhabditis, Diplogaster, Diplogasteroides, Monochoides, Cephalobus, Cylindrocorpus, Turbatrix, examples of the other two Dorylaimus, Aphelenchoides

B General Morphology

Round, slender, nonsegmented (some with markings on outside), most of the free-living forms microscopic in size although dorylaimids up to several mm in length, sex separated but some parthenogenetic, complete alimentray tract with elaborate mouth parts with or without spear (or stylet), no circulatory or respiratory system

C General Physiology

Most sewage treatment plant dwellers feeding on bacteria, others preying on protozoa, small nematodes, rotifers, etc , clean water species vegetarians, DO diffused through cuticle, rhabditids tolerating lower DO than clean water spp, reproduction - eggs - larvae 4 molts) - adults

V ROTIFERS

A Classification.

1 Classified either as a class of the phylum Aschelminthes (various forms of worms) or as a separate phylum (Rotifera), commonly called wheel animalcules, on account of circular appearing movement of cilia around head (corona), corona contracted when crawling or swimming and expanded when attached to catch food.

2 Of the 3 classes, 2 (Seisonidea and Bdelloidea) grouped by some authors under Digononta (2 ovaries) and the other being Monogononta (1 ovary), Seisonidea containing mostly marine forms

3 Class Bdelloidea containing 1 order (Bdelloida) with 4 families, Philodinedae being the most important

4 Class Monogononta comprising 3 orders· Ploima with 14 families, Flosculariaceae with 4 families, and Collothecaceae with 1 family, most important genera included in the order Ploima (i.e., Brachionus, Keratella, Monostyla, Trichocerca, Asplanchna, Polyarthra, Synchaeta, Microcodon), common genera under the order Flosculariaceae Floscularia, Limnias, Conochilus, and Atrochus

5 Unfortunately orders and families of rotifers based on character of corona and trophi (chewing organ), which are difficult to study, esp the latter, the foot and cuticle much easier to study

B General Morphology and Physiology

1 Body weakly differentiated into head, neck, trunk, and foot, separated by folds, in some, these regions are merely gradual changes in diameter of body and without a separate neck, segmentation external only

2 Head with corona, dosal antenna, and ventral mouth, mastax, a chewing organ, located in head and neck, connected to mouth anteriorly by a ciliated gullet and posteriorly to a large stomach occupying much of the trunk.

3 Common rotifers reproducing parthenogenetically by diploid eggs, eggs laid in water, cemented to plants, or carried on femals until hatching.

4 Foot, a prolongation of body, usually with 2 toes, some with one toe, some with one toe and an extra toe-like structure (dorsal spur)

5 Some, like Philodina, concentrating bacteria and other microbes and minute particulate organic matter by corona, larger microbes chewed by mastax, some such as Monostyla feeding on clumped matter, such as bacterial growth, fungal masses, etc at bottom, virus generally not ingested - apparently undetected by cilia.

6 DO requirement somewhat similar to protozoa, some disappearing under reduced DO, others, like Philodina, surviving at as little as 2 ppm DO.

VI SANITARY SIGNIFICANCE

A Pollution tolerant and pollution nontolerant species - hard to differentiate - requiring specialist training in protozoa, nematodes, and rotifers.

B Significant quantitative difference in clean and polluted waters - clean waters containing large variety of genera and species but quite low in densities.

C Aerobic sewage treatment processes (trickling filters and activated sludge processes, even primary settling) ideal breeding grounds for those that feed on bacteria, fungi, and minute protozoa and present in very large numbers, effluents from such processes carrying large numbers of these zoomicrobes, natural waters receiving such effluents showing significant increase in all 3 categories

D Possible Pathogen Carriers

1 Amoebae and nematodes grown on pathogenic enteric bacteria in lab, none alive in amoebic cysts, very few alive in nematodes after 2 days after ingestion, virus demonstrated in nematodes only when very high virus concentrations present, some free-living amoebae parasitizing humans.

2 Swimming ciliates and some rotifers (concentrating food by corona) ingesting large numbers of pathogenic enertic bacteria, but digestion rapid, no evidence of concentrating virus, crawling ciliates and flagellates feeding on clumped organisms.

3 Nematodes concentrated from sewage effluent in Cincinnati area showing live E coli and streptococci, but no human enteric pathogens

VII EXAMINATION OF SEWAGE TREATMENT EFFLUENT, AND SLUDGE FOR MICROBES

A Bacteria - Not Included

B Zoomicrobes -

The 12th edition of the Standard Methods (1965) has a part on Biologic Examination of Water, Wastewater, Sludge, and Bottom Materials, in which the sludge of sewage treatment is discussed, but very briefly. Much of the materials are concerned with sediment at bottom of natural bodies of water Chang described a method for examination of water for nematodes, but the method is not applicable to sewage treatment, sludge, or effluent

1 Waste treatment - the method bound to be qualitative, material scraped from stones in trickling filters or the floc masses in activated sludge examined in slide-coverslip preparations for poor, moderate, or rich zoobiota, material relatively rich in zoobiota indicating satisfactory treatment process, protozoa, rotifers, and nematodes predominant, especially protozoa, bristle worms and watermites in smaller numbers, springtails and insect larvae present as grazing fauna on top of trickling filters

2 Sludge - representative samples suspended in known quantities of dilution water and thoroughly shaken, filtered through bolting cloth or metal screen of comparable pore size to remove extraneous dead clumped matter,filtrate examined in Sedgewick Rafter (SR) counting cell for various zoomicrobes, fresh sludge desired or samples refrigerated

3 Sewage effluent - samples "fixed" with formalin, merthiolate, or similar chemical not desirable for examination for zoomicrobes, 50-200 ml filtered through a 7- or 14-micron membrane and strained material washed with a few mls of dilution and examined in an SR cell for zoo-microbes quantitatively or qualitatively

VIII USE OF ZOOMICROBES AS POLLUTION INDEX

A Idea not new, protozoa suggested long ago, many considered impractical because of the need of identifying pollution-intolerant and pollution-tolerant species - protozoologist required.

B Can use them on a quantitative basis - nematodes, rotifers, and nonpigmented protozoa present in small numbers in clean water Numbers greatly increased when polluted with effluent from aerobic treatment plant or recovering from sewage pollution, no significant error introduced when clean-water members included in the enumeration if a suitable method of computing the pollution index developed

C Most practical method involves the equation (A + B)/A = Z.P.I., where A = number of pigmented protozoa, B = other zoomicrobes, in a unit volume of sample, and Z.P.I. = zoological pollution index. For relatively clean water, the value of Z P I. close to 1, the larger the value above 1, the greater the pollution by aerobic effluent, or sewage during recovery This is based on the fact that pigmented protozoa are members of clean water micro-fauna (stabilization pond excluded)

IX CONTROL

A Chlorination of Effluent and Settling

B Prolongation of Detention Time of Effluent

C Modification of Waste Treatment

D Elimination of Slow Sand Filters in Nematode Control

X LIST OF COMMON ZOOLOGICAL ORGANISMS FOUND IN SEWAGE TREATMENT PROCESS - TRICKLING FILTERS AND ACTIVATED SLUDGE PROCESS

PROTOZOA

Sarcodina - Amoebae

- Amoeba proteus, A radiosa
- Hartmanella spp.
- Arcella vulgaris
- Naegleria gruberi
- Actinophrys sol

FLAGELLATA

- Bodo caudatus
- Pleuromonas jaculans
- Oikomonas termo
- Cercomonas longicauda
- Peranema trichophorum

Swimming type

Ciliophora

- Colpidium colpoda
- Colpoda cucullus
- Glaucoma
- Paramecium caudatum; P. bursaria

Stalked type

- Opercularia spp. (short stalk dichotomous)
- Vorticella spp. (stalk single and contractile)
- Epistylis plicatilis (like Opercularia more colonial)
- Carchesium spp. (like Vorticella but colonial, both have spiral coiled stalk when contracted)

Crawling type

- Euplotes spp.
- Stylonychia mylitus
- Urostyla spp.
- Oxytricha spp.

NEMATODA

- Diplogaster spp.
- Monochoides spp.
- Diplogasteroides spp.
- Rhabditis spp.
- Pelodera spp.
- Aphelenchoides sp.
- Dorylaimus sp.

Cylindrocorpus sp.
Cephalobus sp.
Rhabdolaimus sp.
Monhystera sp.
Trilobus sp.

ROTATORIA

Diglena
Monstyla
Polyarthra
Philodina
Keratella
Brachionus

OLIGOCHAETA (bristle worms)

Aelosoma hemprichi (Aelosomatidae)
Aulophorus vagus (Naididae)
Tubifex tubifex (Tubificidae)
Pachydrilus lineatus (Enchytraeidae)

INSECT LARVAE

Metriocnemus ssp. (midge)
Orthocladius ssp. (midge)
Psychoda spp. (filter fly)

OTHER ARTHROPODA

Hydrochna sp. (Acarina, mite)
Platysieus tenuipes (Acarina, mite)
Hypogastrura (= *Achorutes sub-viatica viaticus*) (Collembola, Springtail)
Folsomia sp. (Collembola, Springtail)
Tomocerus sp. (Collembola, Springtail)

MOLLUSCA

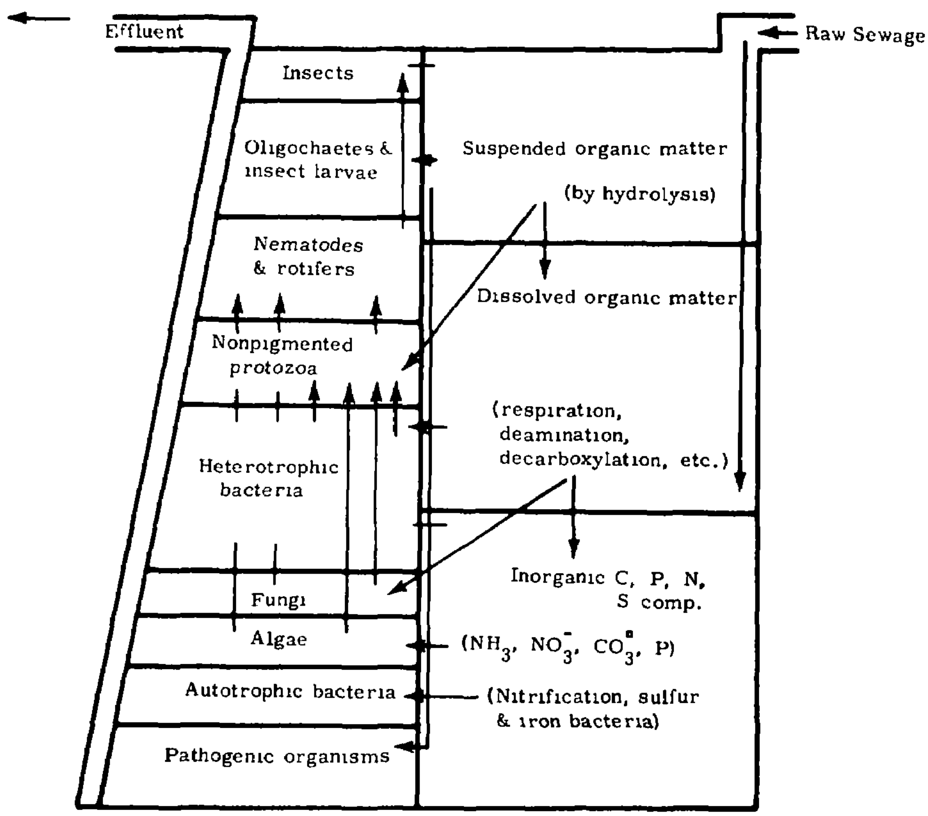
Lymnaea ssp. (pulmonate snail)
Physa sp. (pulmonate snail)

XI POPULATION DYNAMICS AND THE FOOD CHAIN IN AEROBIC SEWAGE TREATMENT PROCESSES (Figure 1 and 2)

A Aerobic bio-oxidation of waste materials comparable to a food chain through which the dead organic matter is converted to inorganic matter during the stabilization process, e.g., waste organic matter ⟶ bacterial phase ⟶ zoological phase ⟶ inorganic matter.

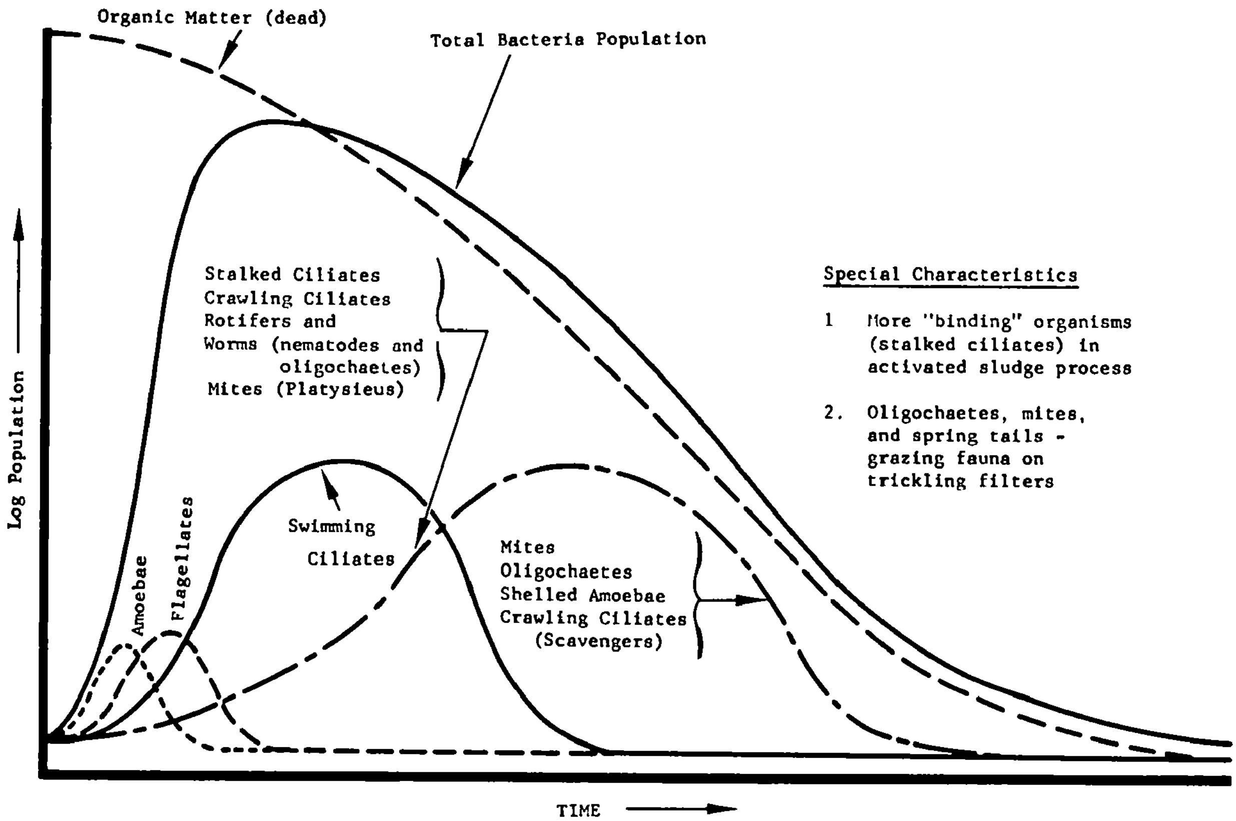
B Systematics, physiology and biochemistry involved in explaining the "chain reaction", knowledge inadequate and fragmental, ecological study limited to principles governing the relationship of different groups of flora and fauna with each other and with the environment

C With adequate DO supply, bacterial population increases rapidly in the presence of rich organic food, flagellates and amoebae, which feed on bacteria and other small particulate matter in clumped material (such as growth film and floc masses), first show increase in population size, as suspended bacterial population increases to a high level, swimming ciliates, which feed actively on the suspended bacteria, also increase, increased consumption of bacteria and reduced supply of dead organic matter results in decline in bacterial population, which, in turn, results in a decline in the swimming ciliate population, the presence of large populations of small protozoa (ciliates, flagellates, and amoebae) results in an increase in populations of rotifers, nematodes, stalked ciliates, and crawling ciliates, which feed on the small protozoa and bacteria that are lodged in clumped masses, eventually, scavengers, such as mites, shelled amoebae, certain nematodes, and bristle worms become predominant, and bacteria and small protozoa populations drop to the pre-cycle level, rotifers that can concentrate bacteria in suspension, (such as *Rotifer* and *Philodina*, and nematodes), which have long surviving time, may remain for a long time; these zoomicrobes appear in the effluent in proportion to their respective population during treatment - nematodes, rotifers, ciliates predominant with small numbers of flagellates and amoebae, bristle worms, unpredictable, mites few



Food Chain in Aerobic Sewage Treatment Processes

Figure 1.



Population dynamics in Aerobic Sewage Treatment Process

Figure 2

D Since sewage effluent from aerobic treatment processes are rich in nonpigmented zoomicrobes, discharge of effluent into natural causes great increase in their members, unpolluted waters usually have a much higher algae-to-nonpigmented-zoomicrobes ratio The great increase in the latter in water resulted from effluent pollution is likely to change this ratio, thus giving the basis for the Z.P.I. This analysis is not applicable to stabilization ponds due to the large algal population present in their effluents

Microbial Agents

- Utilization
  - Treatment Process
    - Stabilization of Organic Matter
      - Aerobic
      - Anaerobic
    - Breakdown of Specific Industrial Wastes
    - Stabilization Ponds
      - Aerobic and Anaerobic Combined
    - Nitrification - Aerobic
      - Dentrification when Anaerobic
    - Elimination of Nitrogen Through
      - N_2 Formation - Special Dentrification
    - Sludge Digestion
      - Aerobic
      - Anaerobic
    - Phosphorus Removal
      - Essential in Preventing Algae Growth
    - (Composting) - Solid Wastes
  - Pollution Indicator
    - Total Bacteria Count
      - Coliform Density
        - MF Counts
        - MPN Tube Tests
      - E. Coli Density
        - Tube Test
        - MF Counts
      - Streptococci
        - Tube Test
        - MF Counts
      - Infrared Spectrophotometer
        - Specificity
        - Time Requirement
      - Enteric Pathogens
        - Salmonella Spp.
          - Quantitation in Mixed Population
        - Enteroviruses
          - Large Volumes Required
      - Coli Phage
        - Type Specificity To Be Considered
      - Fluorescent Antibody
        - Against E. Coli
        - Against Enteric Pathogens
      - Gas Chromatography on Metabolic Products
      - Zoological Pollution Index
- Elimination
  - Removal
    - Detention
    - Settling
      - Limited to Large Organisms
    - Flocculation
      - Alum
      - Iron
      - Lime
      - "Coagulant Aid"
    - Filtration
      - Adsorption
      - Straining
      - Medium
      - Reverse Osmosis
    - Metal Strainer
      - Mesh Size Limit
    - Antagonistic Agent
      - Pseudomonads
    - Bacterial Feeders
      - Protozoa
      - Nematodes
      - Rotifers
    - Flotation
      - Aeration
      - Anionic Detergent
      - Neutral Detergent
      - Cationic Detergent
  - Destruction or Disinfection
    - Chemical
      - Clorine
      - Bromine
      - Iodine
      - Ozone
      - Silver
      - Copper
    - Physical
      - Heat
      - UV
      - Gamma
      - Radiation
      - Wave Motion
      - Electro-Hydraulic
    - Treatment

Figure 3

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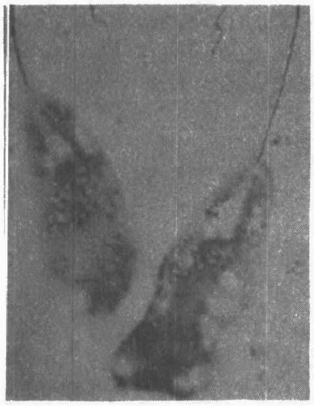
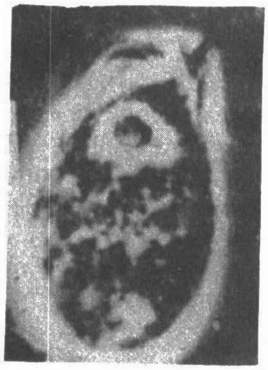
This outline was prepared by S. L. Chang, M. D., Chief, Etiology, Division of Water Supply Programs Division, WPO, EPA. Revised by R. M. Sinclair, Aquatic Biologist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

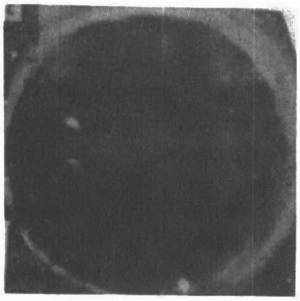


a. Peranema trichoporum, 25u

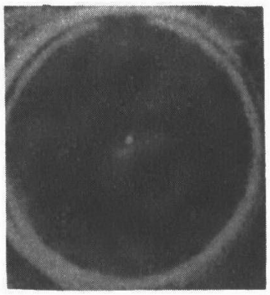
b. Naegleria gruberi amoeba stage, 18u



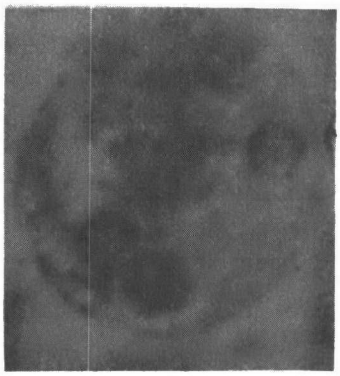
c. N. gruberi flagellate stage



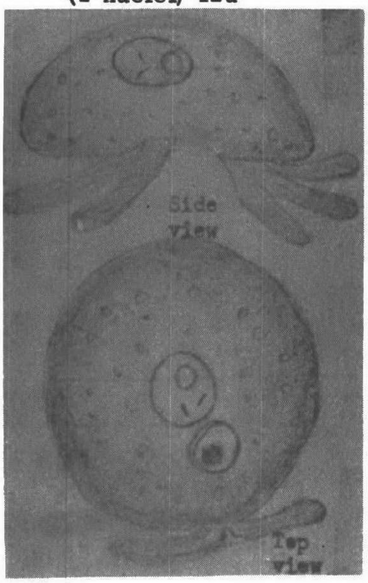
d. N. gruberi, cyst (2 nuclei) 12u



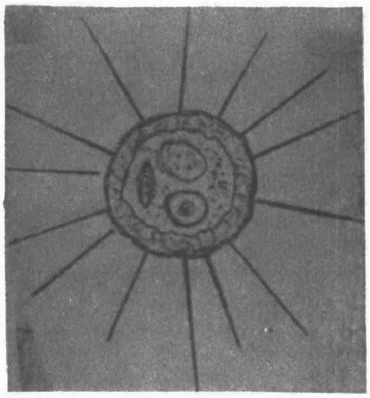
e. N. gruberi, cyst (1 nucleus)



f. Endamoeba histolytica cyst (4 nuclei) 16u



g. Arcella vulgaris 40u

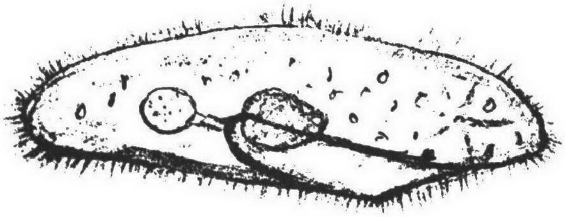


h. Actinosphaerium sp. 200-300u (with a navicula inside)

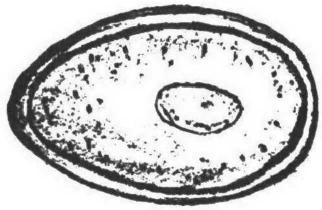
i. Bodo caudatu 10-20u

Fig. 1

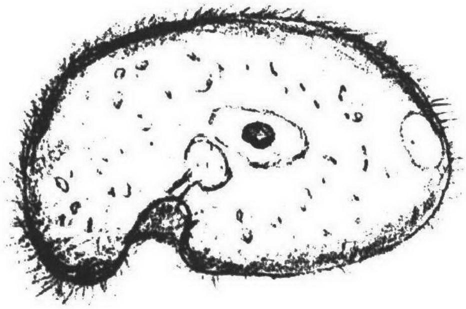
S. L. Chang, 1963



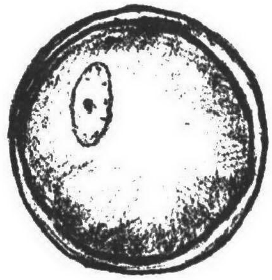
a. Paramecium caudatum
200 - 260u



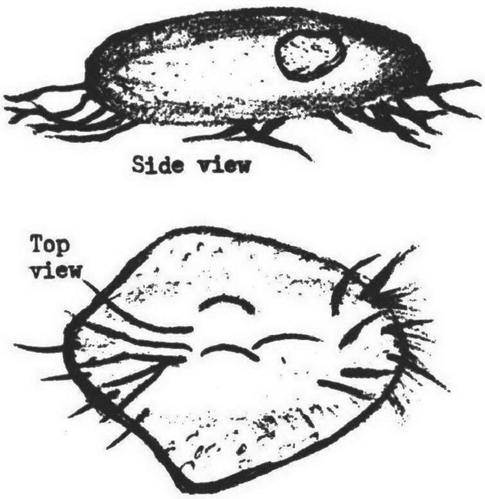
b. P. caudatum
cyst



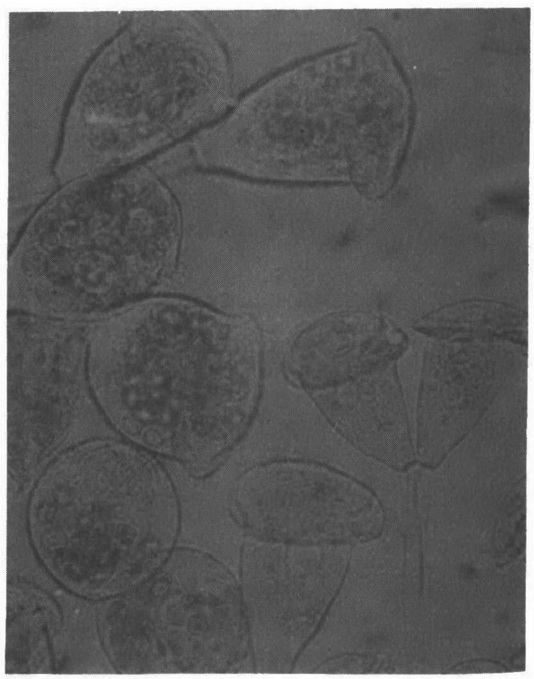
c. Colpoda sp. 20-120u



d. Colpoda cyst



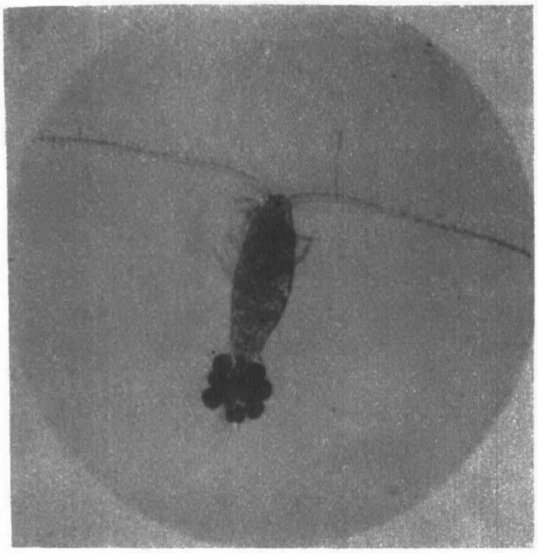
e. Euplotes carcinatus
70u



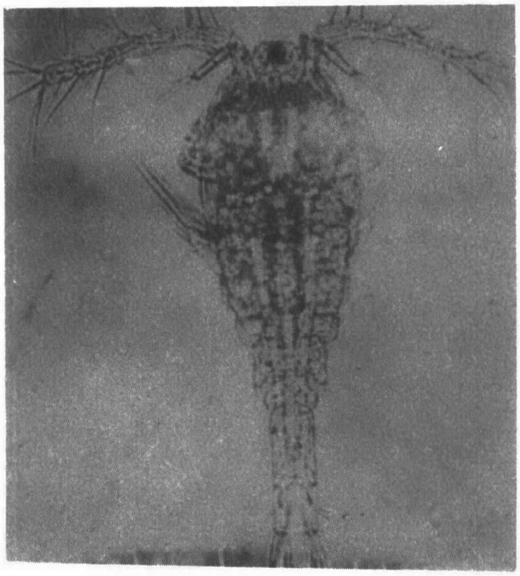
f. Vorticella 35-157u

Fig. 2

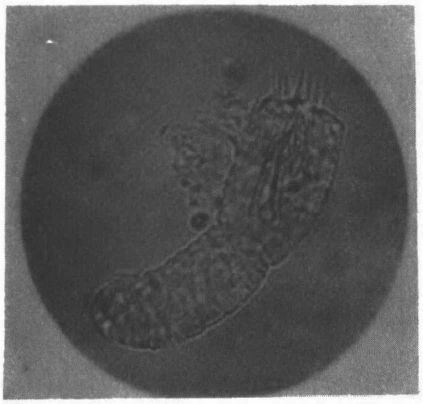
S. L. Chang, 1963



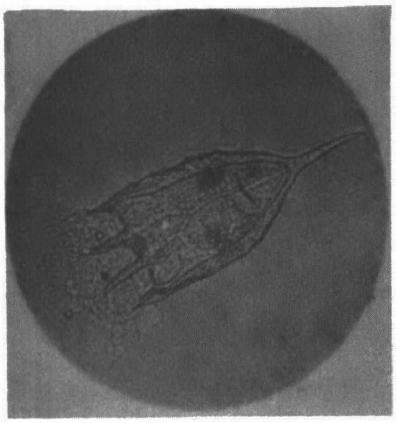
a. Diaptomus sp. 2 mm.
(2 egg sacs



b. Cyclops sp. 2 mm.

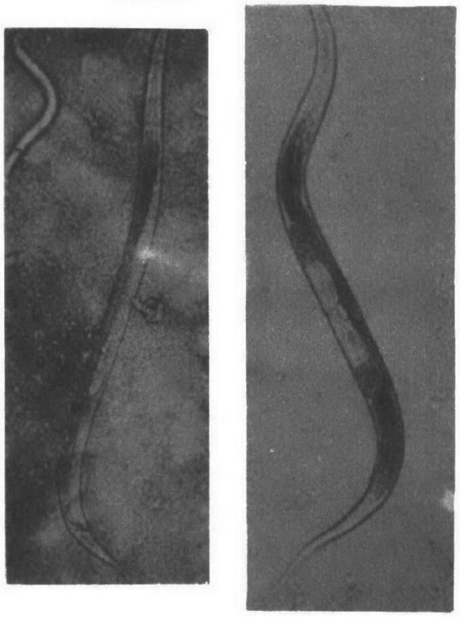


c. Philodina sp. 45u



d. Anurea cochlearis 125u

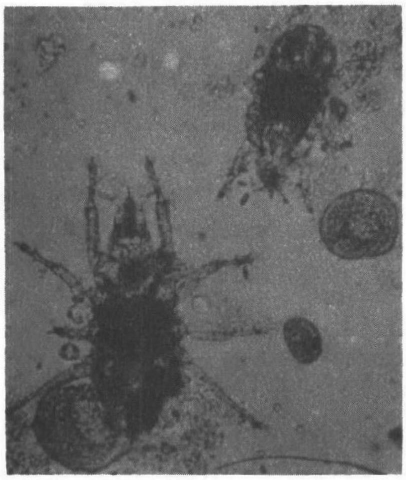
larva



male female

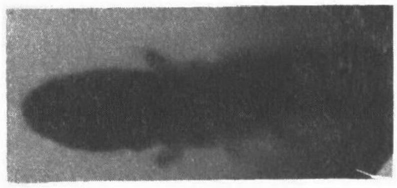
e. Diplogaster nudicapitatus
about 1 mm.

Fig. 3 S. L. Chang, 1963

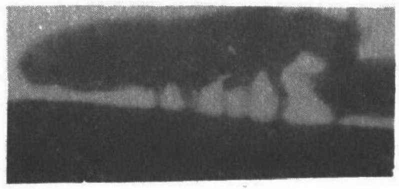


a. Hydrochna sp. (water mite) (50X)

Dorsal view

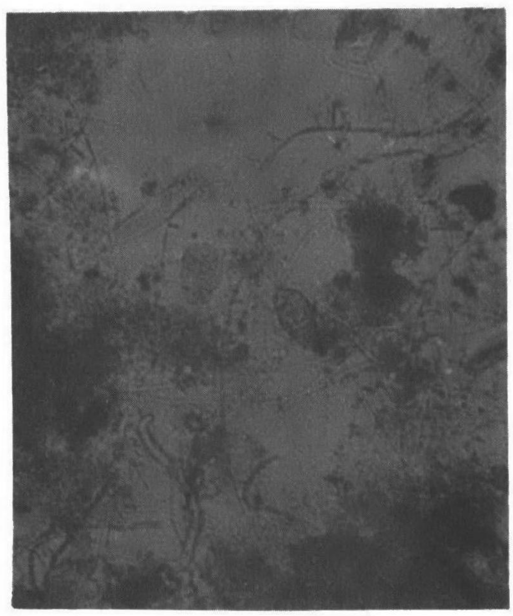


b. Folsomia fimetaria (50X)



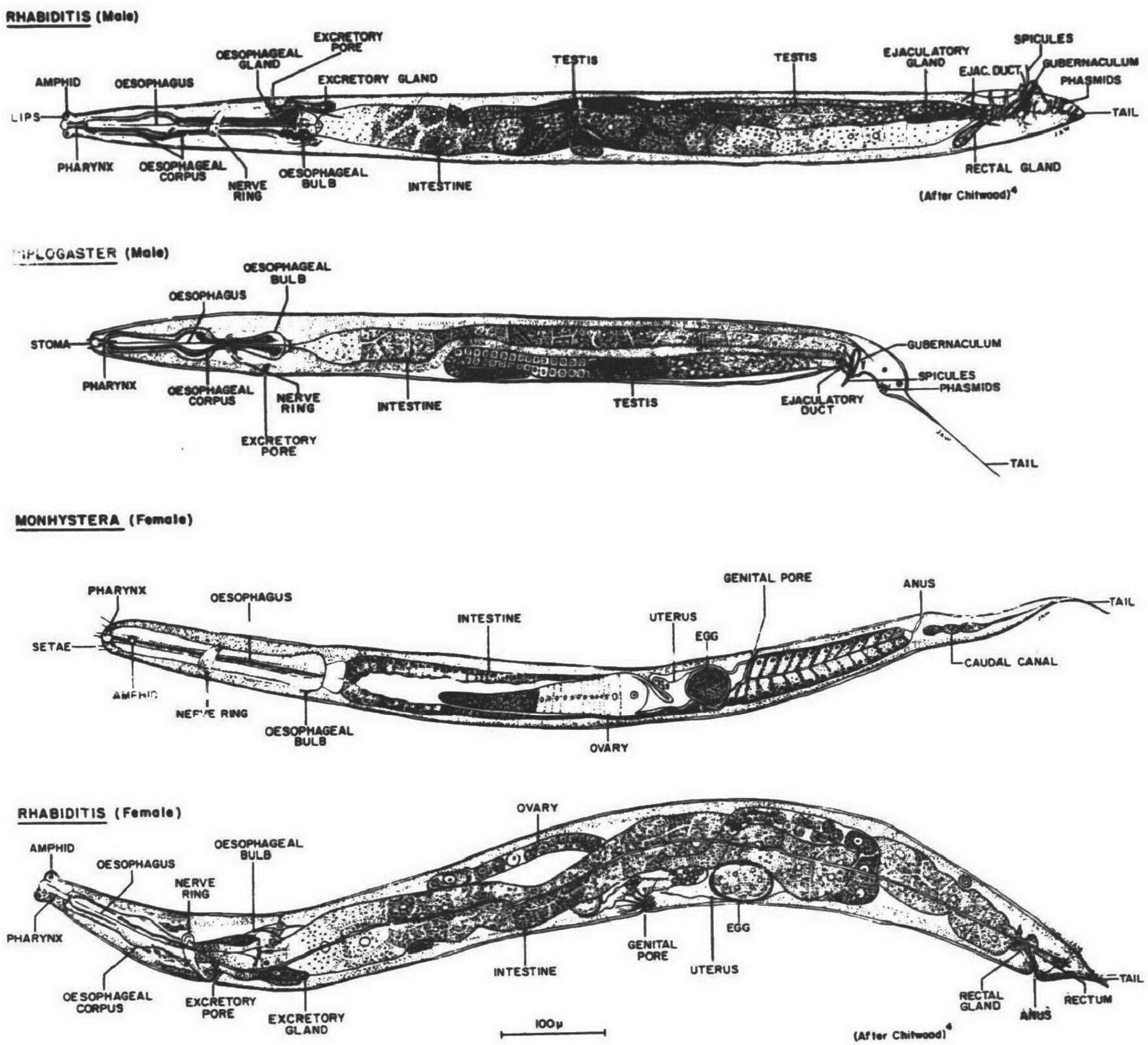
c. F. fimetaria (side view)

d. Typical zoological organisms found in growth mass in Trickling filters (50X)



e. Typical zoological organisms found in floc masses in activated sludge process (50X)

Fig. 4 **S. L. Chang, 1963**



Free Living Nematodes

(Nemathelminthes)

BIOLOGY OF ZOOPLANKTON COMMUNITIES

I CLASSIFICATION

A The planktonic community is composed of organisms that are relatively independent of the bottom to complete their life history. They inhabit the open water of lakes (pelagic zone). Some species have inactive or resting stages that lie on the bottom and carry the species through periods of stress; e. g., winter. A few burrow in the mud and enter the pelagic zone at night, but most live in the open water all the time that the species is present in an active form.

B Compared to the bottom fauna and flora, the plankton consists of relatively few kinds of organisms that are consistently and abundantly present. Two major categories are often called phytoplankton (plants) and zooplankton (animals), but this is based on an outmoded classification of living things. The modern tendency is to identify groupings according to their function in the ecosystem: Primary producers (photosynthetic organisms), consumers (zooplankton), and decomposers (heterotrophic bacteria and fungi).

C The primary difference then is nutritional, phytoplankton use inorganic nutrient elements and solar radiation. Zooplankton feed on particles, much of which can be phytoplankton cells, but can be bacteria or particles of dead organisms (detritus) originating in the plankton, the shore region, or the land surrounding the lake.

D The swimming powers of planktonic organisms is so limited that their horizontal distribution is determined mostly by movements of water. Some of the animals are able to swim fast enough that they can migrate vertically tens of meters each day, but they are capable of little horizontal navigation. At most, some species of crustaceans show a general avoidance of the shore areas during calm weather when the water is moving more slowly than the animals can swim. By definition, animals that are able to control their horizontal location are nekton, not plankton.

E In this presentation, a minimum of classification and taxonomy is used, but it should be realized that each group is typified by adaptations of structure on physiology that are related to the planktonic mode of existence. These adaptations are reflected in the classification.

II FRESHWATER ZOOPLANKTON

A The freshwater zooplankton is dominated by representatives of three groups of animals, two of them crustaceans· Copepoda, Cladocera, Rotifera. All have feeding mechanisms that permit a high degree of selectivity of food, and two can produce resting eggs that can withstand severe environmental conditions. In general the food of usual zooplankton populations ranges from bacteria and small algae to small animals.

B The Copepoda reproduce by a normal biparental process, and the females lay fertilized eggs in groups which are carried around in sacs until they hatch. The immature animals go through an elaborate development with many stages. The later stages have mouthparts that permit them to collect particles. In many cases, these are in the form of combs which remove small particles by a sort of filtration process. In others, they are modified to form grasping organs by which small animals or large algae are captured individually.

C The Cladocera (represented by Daphnia) reproduce much of the time by parthenogenesis, so that only females are present Eggs are held by the mother in a brood chamber until the young are developed far enough to fend for themselves. The newborn animals look like miniature adults, and do not go through an elaborate series of developmental stages in the water as do the copepods. Daphnia has comb-formed filtering structures on some of its legs that act as filters.

D Under some environmental conditions the development of eggs is affected and males are produced. Fertilized eggs are produced that can resist freezing and drying, and these carry the population through unsatisfactory conditions.

E The Rotifera are small animals with a ciliated area on the head which creates currents used both for locomotion and for bringing food particles to the mouth. They too reproduce by parthenogenesis during much of the year, but production of males results in fertilized, resistant resting eggs. Most rotifers lay eggs one at a time and carry them until they hatch.

III ZOOPLANKTON POPULATION DYNAMICS

A In general, zooplankton populations are at a minimum in the cold seasons, although some species flourish in cold water. Species with similar food requirements seem to reproduce at different times of the year or are segregated in different layers of lakes.

B There is no single, simple measurement of activity for the zooplankton as a whole that can be used as an index of production as can the uptake of radioactive carbon for the phytoplankton. However, it is possible to find the rate of reproduction of the species that carry their eggs. The basis of the method is that the number of eggs in a sample taken at a given time represents the number of animals that will be added to the population during an interval that is equal to the length of time it takes the eggs to develop. Thus the potential growth rate of the populations can be determined. The actual growth rate, determined by successive samplings and counting, is less than the potential, and the difference is a measure of the death rate.

C Such measurements of birth and death rates permits a more penetrating analysis to be made of the causes of population change than if data were available for population size alone.

D Following is an indication of the major environmental factors in the control of zooplankton.

1 Temperature has an obvious effect in its general control of rates. In addition, the production and hatching of resting eggs may be affected.

2 Inorganic materials

Freshwater lakes vary in the content of dissolved solids according to the geological situation. The total salinity and proportion of different dissolved materials in water can affect the population. Some species are limited to soft water, others to saline waters, as the brine shrimp. The maximum population size developed may be related to salinity, but this is probably an indirect effect working through the abundance of nutrients and production of food.

3 Food supply

Very strong correlations have been found between reproduction and food supply as measured by abundance of phytoplankton. The rate of food supply can affect almost all aspects of population biology including rate of individual growth, time of maturity, rate of reproduction and length of life.

4 Apparently in freshwater, dissolved organic materials are of little nutritional significance, although some species can be kept if the concentration of dissolved material is high enough. Some species require definite vitamins in the food.

5 Effect of predation on populations

The kind, quantity and relative proportions of species strongly affected by grazing by vertebrate and invertebrate predators. The death rate of Daphnia is correlated with the abundance of a predator. Planktivorous fish (alewives) selectively feed on larger species, so a lake with alewives is dominated by the smaller species of crustaceans and large ones are scarce or absent.

6 Other aspects of zooplankton

Many species migrate vertically considerable distances each day. Typically, migrating species spend the daylight hours deep in the lake and rise toward the surface in late afternoon and early evening.

Some species go through a seasonal change of form (cyclomorphosis) which is not fully understood It may have an effect in reducing predation.

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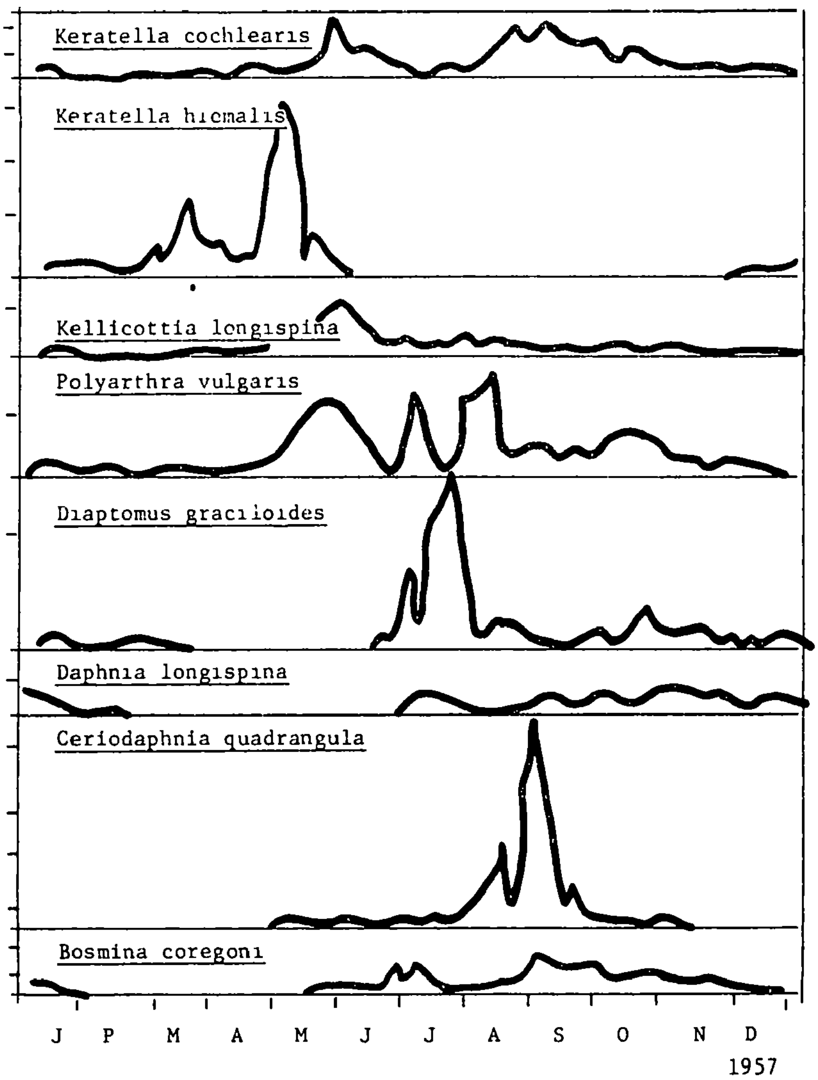
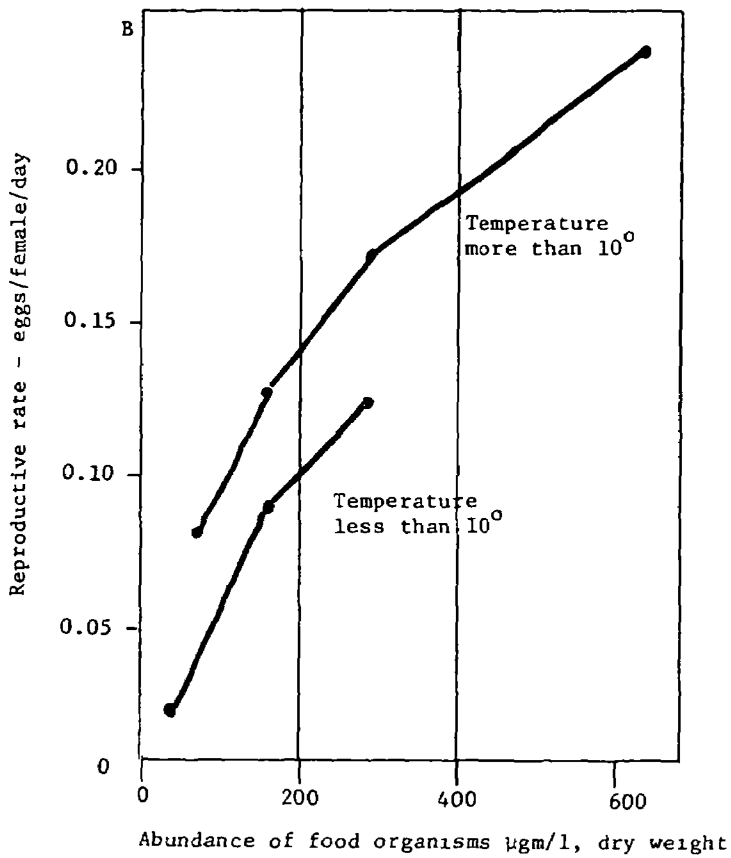
This outline was prepared by W. T. Edmondson, Professor of Zoology, University of Washington, Seattle, Washington.

FIGURE 1 SEASONAL CHANGES OF ZOOPLANKTON IN LAKE ERKEN, SWEDEN

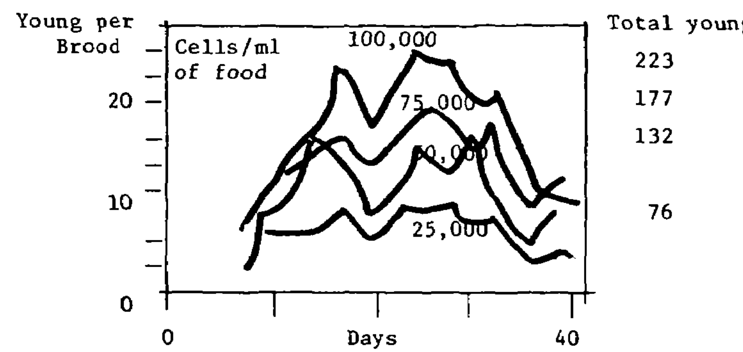


Each panel shows the abundance of a species of animal. Each mark on the vertical axis represents 10 individuals/liter. Nauwerck, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken. Symbolae Botanicae Upsaliensis, 17:1-163.

FIGURE 2 REPRODUCTIVE RATE OF ZOOPLANKTON AS A FUNCTION OF ABUNDANCE OF FOOD

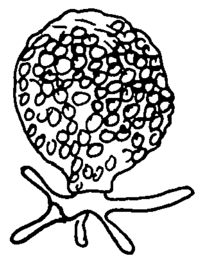


Mean rate of laying eggs by the planktonic rotifer <u>Keratella cochlearis</u> in natural populations as a function of abundance of food organisms and temperature. W. T. Edmonson. 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol. Mmogr. 35. 61-111.



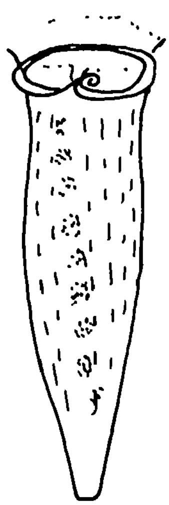
Number of young produced in each brood by Daphnia living in four different concentrations of food organisms, renewed daily. The total number produced during the life of a mother is shown by the numbers at the right. The Daphnia at the two lowest concentrations produced their first batch of eggs on the same day as the others, but the eggs degenerated, and the first viable eggs were released two days later. Richman, S. 1958. The transformation of energy by <u>Daphnia pulex</u>. Ecol. Monogr. 28: 273-291.

PROTOZOA

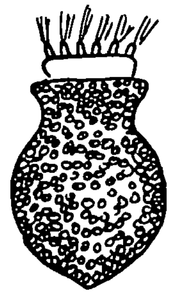


Difflugia

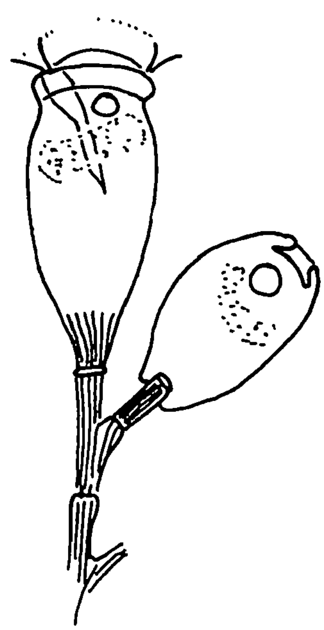
Amoebae



Stentor



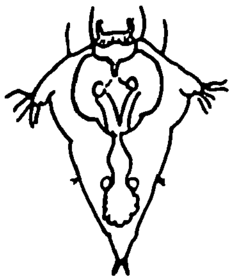
Codonella



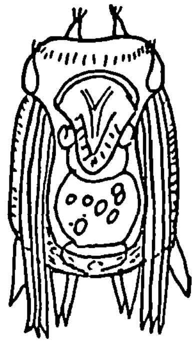
Epistylis

Ciliates

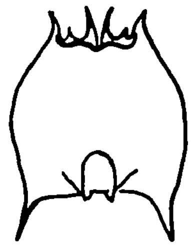
ROTIFERA



Synchaeta



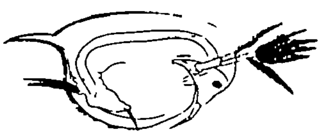
Polygarthra



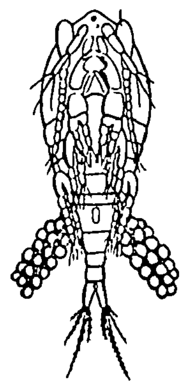
Brachionus

ARTHROPODA

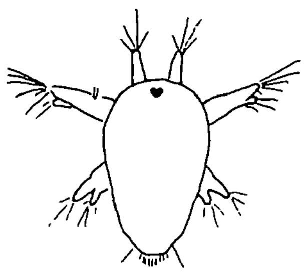
Crustacea



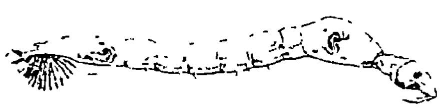
Cladocera



Copepoda

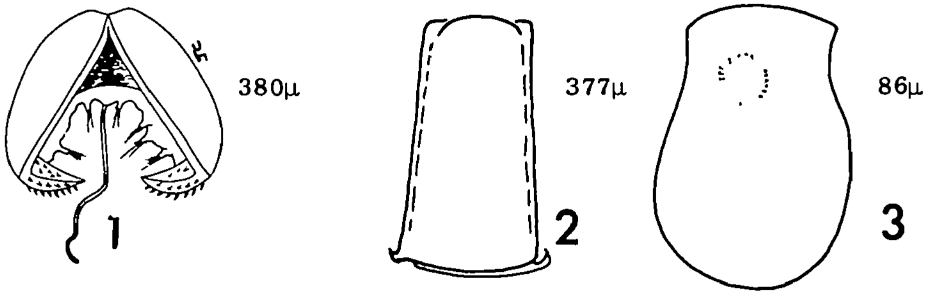


Nauplius larva of copepod



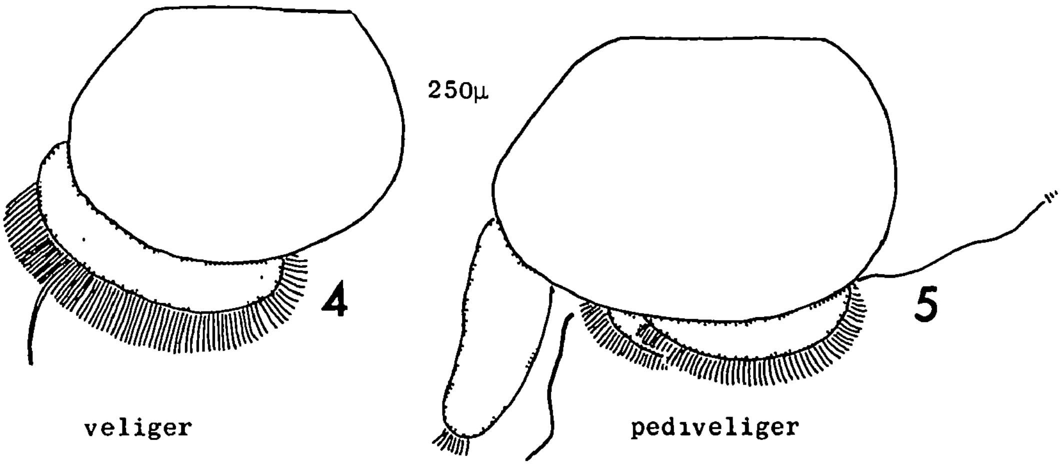
Insecta - Chaoborus

PLANKTONIC BIVALVE LARVAE



spined (fin attached) simple (gill attached)

Glochidia (Unionidae) Fish Parasites
(1-3)



Veliger Larvae (Corbiculidae) Free Living Planktonic
(4-5)

Pediveliger attaches byssus lines)

MACRO INVERTEBRATES

I INTRODUCTION

Groups included are in general those which may be seen and recognized without the use of a microscope For a more restricted definition in reference to bottom sampling, they are defined as those invertebrates retained on a No. 30 sieve (approx. 0.5 mm aperture).

II PHYLUM PORIFERA - Sponges

A Often encountered in the "pipe-moss" complex. Being true animals, they will grow in the dark and hence require only a water possessing adequate food materials.

B Freshwater sponges usually appear as brownish or greenish masses, (where containing zoochlorellae), irregular in shape, growing on twigs or solid surfaces. Erect or branching shapes sometimes found. Surface non-shiny, texture delicate. May form overgrowths on irrigation canal walls.

C Microscopic structure characterized by silica "spicules" and reproductive structures known as "gemmules"

1 Spicules are in general long slender crystals In some, the ends are simple or pointed, in others expanded into various shapes

2 Spicules of various types are interwoven like the twigs of a bird nest to form the skeleton of the sponge The nature of the soft living tissue cells indicate a probable evolutionary origin of the sponge from flagellated and amoeboid protozoa

3 Gemmules are little bundles of tissue cells, protected by spicules, which can resist unfavorable conditions They are often found scattered throughout the mass of a sponge These are analagous to turions in some aquatic plants and statoblasts in bryozoans.

III PHYLUM COELENTERATA - The Jellyfishes, Corals, and Hydras

A This group is of relatively little importance in fresh water, although quite prominent in the ocean.

B The freshwater hydra is a typical and simple coelenterate Its structure is essentially a long slender sac, two layers thick, with tentacles extending out from around the open end or mouth

1 Fully extended individuals may measure a half inch or more

2 Generally an indicator of clean water. Rarely a nuisance

C Craspedacusta, a freshwater jellyfish occasionally appears in great numbers in lakes and reservoirs in late summer. No particular nuisance conditions are known to result, although tastes and odors might be expected if sufficient numbers were taken into a water treatment plant No reasons are known for their sporadic appearances, no public health significance is known, and no control measures can be recommended.

D Cordylophora, a colonial hydroid is a typical attached organism in large rivers, lakes, and reservoirs.

IV PHYLUM PLATYHELMINTHES - Tapeworms, Flukes, and Planarias

A Tapeworms and flukes are serious human parasites in many parts of the world, but generally under good control in North America. Most of them have relatively complicated life histories involving one or more "intermediate" hosts and a "final" host

B The human tapeworm Diphyllobothrium latum is a form of modest significance, endemic to our northern states.

1 It is the largest of the human tapeworms

2 It is obtained by eating underdone fish of the pike family

C Human fluke parasites have certain species of snails as intermediate hosts Larval forms, cercariae released from snails penetrate human skin directly while the person is wading or bathing in infested waters.

1 Although one or two species of snail in southern United States are thought to be capable of transmitting the human blood fluke, none are known to do so at the present time.

2 Flukes also parasitize other animals than man. Occasionally the cercaria larvae of non-human parasites will be attracted to humans bathing in infested waters. They are able to enter the skin but cannot complete penetration, and so are trapped. The result is a rash, often quite painful, known as "swimmer's itch". This now occurs widely across the northern states and in many coastal waters. Control measures are directed at the elimination of species of snails.

D The Planarians (Class Turbellaria) are large enough to spot with the naked eye. They are useful field indicators of pollution, but shrivel up on preservation, and are seldom recognized in the laboratory.

V PHYLUM BRYOZOA - Moss Animals

A These are small, colonial, sessile animals, common in both marine and fresh waters. Their main significance is as contributors to the pipe-moss complex, and as indicators of the degree of pollution.

B Freshwater forms are usually either creeping brown moss-like forms that grow over the undersides of rocks or in pipes, or larger gelatinous masses growing on sticks and rocks in lakes and reservoirs.

1 The closely adhering threads on rocks or pipes may range up to 1/16th of an inch in width. Microscopic examination reveals numerous raised openings from which when undisturbed, tiny fans of tentacles (the lophophore) are extended. Closely packed colonies may reach an inch in depth, and if adjacent colonies have come into contact, an indefinite area may be covered.

2 Another type produces clear jelly-like masses of transparent or faintly tinted material, with minute, often colored, individuals (zooids) scattered over the surface. The animals are extremely timid and only with great care and patience can they be observed with the tentacles expanded. Colonies of some species are reported to approach six feet in length, but smaller forms are more common. Certain types of colonies can slowly change their position.

3 Reproduction is by means of unique structure known as a "statoblast". This is a discoid or eleptical structure, often with anchor-shaped hooks, which can resist winter conditions and even drying. Statoblasts usually germinate in spring, colonies reach full development by late summer.

VI PHYLUM MOLLUSCA - Snails, Clams, also Oysters, Squids, Octopi

A Freshwater molluscs are in general animals with a soft body, encased in a calcareous shell which may be single (snails) or double (clams and mussels). Marine forms such as the squid, nautilus, octopus, slugs, and others, would require additional qualification.

B In the snail group (Class Gastropoda) the shell may be coiled in various ways, or a simple tent-shaped secretion on the animal's back. These animals possess a distinct head, with a pair of contractile tentacles, at the base of which are placed the eyes.

1 The mouth is provided with a unique flexible rasp-like structure, the radula. Chitinous jaws too are usually present.

2 The two main groups in freshwater are the air breathers (Order Pulmonata) and the water breathers (Order Streptoneura). Since all Streptoneura have a peculiar chitinous or calcareous "trap door" called an operculum (used for closing the shell) they are also called the "operculate" snails (vs the "nonoperculate" Pulmonata).

3 Many snails are classed as "nuisance organisms".

a Snails are quick to take advantage of organic enrichment. As pollution eliminates predators, pulmonate snails (such as Physa, Lymnea) thrive. Trickling filters, polluted streams, and similar locations are often nearly choked with these organisms.

b Certain snails are also the intermediate host for certain fluke parasites as mentioned elsewhere, and hence may constitute an important link in the control of these parasites.

C The Bivalved Molluscs (Class Bivalvia) have the body protected by two symmetrical, opposing valves or shells, which are united above by a flexible elastic tissue called the "ligament," which is also secreted by the mantle.

1 They have no head. The foot is an axe-shaped mass of muscular tissue which may be extended and used to drag the animal ahead. The shell is secreted by two sheets of tissue called the mantle.

2 They feed by straining particles out of the water by means of two sets of lace-like gills (ctenidia). They are thus animated filters and when present in significant numbers may contribute to the reduction of turbidity with resulting solids accumulation.

3 Certain thick shelled forms such as the Unionidae formerly commercially harvested for use in making pearl buttons, are exported to Japan for production of nuclei for the cultured pearl industry.

4 Certain small types (family Sphaeriidae) such as Sphaerium the fingernail clam, have been shown to tolerate considerable organic pollution.

5 The Asian Clam, Corbicula. An exotic, intermediate insize between the above two families, is a serious pipe clogging organism. Unlike the endemic families it has planktonic larvae, hence, it's nuisance potential.

VII PHYLUM ANNELIDA - The segmented Worm Earthworms, Sludgeworms, and Leeches (Sometimes regarded as separate phyla)

A Class Oligochaeta, the earthworm-sludgeworm group. Body clearly divided into segments. Bristle like "setae" or hairs present on most segments, are used in locomotion; in some species may be withdrawn beneath body surface.

1 Accurate identification requires simple clearing procedures with the specimen Although these worms are hermaphroditic (having both sexes in the same individual), many of the smaller forms commonly reproduce by a type of a sexual budding which produces chains of two or more individuals.

2 Aquatic earthworms and sludgeworms, like their terrestrial counterparts, feed on the soil or mud in which they live, and contribute very significantly to its stabilization. Having hemoglobin in their blood, some of them can tolerate very low oxygen tensions. Like the snails mentioned above, they thus thrive in polluted conditions in the absence of predators (ex Tubifex, Limnodrilus.) Smaller types (ex. Aelosoma, Chaetogaster) may abound in activated sludge.

B Class Hirudinea - leeches

1 These organisms are essentially ectoparasites of vertebrates, though they may also feed on smaller annelids, snails, insect larvae, and organic ooze. They are characterized by the possession of an anterior and posterior sucker

disc, and the absence of setae. Eyes if present, are located on the (smaller) anterior or oral sucker, although sensory cells are widely scattered over the general body surface.

2 They are not known to be the vectors of any human disease, although when present in numbers, their blood sucking habits give them a considerable nuisance value.

3 Their tolerance for sewage pollution is considerable and they are hence often present in great numbers in polluted streams.

VIII PHYLUM ARTHROPODA - The Jointed Animals

A Characterized in general by paired jointed legs on a body nearly always segmented, and a chitinous exoskeleton. Three of the major groups have freshwater representatives The Crustacea, Arachnida, and Insecta. The Insecta will be treated in a separate section.

B Class Crustacea

1 Characterized by two pair of antennae, respiration by means of blood gills (or general body surface). The vast majority of crustacea are aquatic. Crabs and lobsters are well known marine examples, water fleas and copepods well known freshwater examples. No freshwater species approach the giant marine species for size where the king crab, for example, may have a leg spread of several feet.

2 A few specialized terms used frequently in connection with the Crustacea are defined below.

Head:	The anterior part of the body containing the mouth. Usually consists of two or more fused segments, each represented by a pair of specialized mouthparts.
Thorax	The major section of the body behind the head. Contains most of the body organs and usually the walking (or swimming) legs.
Abdomen·	The most posterior section of the body. Contains the anus and often gills. Is seldom involved in locomotion except in swimming forms.
Caphalothorax	The fused head and thorax.
Carapace.	A fold of the body wall or shell which usually extends down over each side of the thorax. May cover the whole side or only the bases of the legs.
Antenna	Sensory appendages or "feelers". Typically two in number in the Crustacea.
Biramous.	Two branched.

3 Subclass Branchipoda - phyllopods

These organisms have many pairs of flattened appendages serving for both locomotion and respiration.

The first three orders as listed below tend to inhabit temporary pools, and so are often good indicators of such water. Life histories may often be completed in 2-3 weeks. Occurrence is quite sporadic. Many of them are tolerant of highly saline or alkaline waters.

The Cladocera, the last order, is more of an inhabitant of permanent bodies of water. All are in general, plankton and detritus feeders, often tolerant of high organic content as long as aerobic conditions are maintained.

a Order Anostraca - fairy shrimps. Eleven to 17 pairs of thoracic appendages, elongate, cylindrical body without a carapace, eyes stalked.

Ex Artemia (the brine shrimp), General size range 15-30 mm. extreme 5-100 mm. Swim gracefully on their backs.

b Order Notostraca - tadpole shrimps. Forty - 60 pairs of thoracic appendages, body depressed and partly covered by a dorsal shield like carapace. Eyes sessile. Size up to 100 mm.

c Order Conchostraca - clam shrimps. Ten - 28 thoracic appendages. Body laterally compressed and completely enclosed in a bivalved carapace which is often relatively thin and marked by successive lines of growth. Generally favored by warmer water. Size 4-16 mm.

d Order Cladocera - water fleas. Four 6 pairs of thoracic appendages. Body laterally compressed, all except the the distinct head usually enclosed in a bivalved carapace. Second antenna is branched, and used for locomotion. Single compound eye. Size 0.2-3.0 mm or more. Common genera include Daphnia and Bosmina.

1) This is a large and widely dispersed group, a common component of our plankton in nearly all types of water.

2) Generally parthenogenetic, until unfavorable conditions stimulate the production of males. Sexual eggs result which can withstand freezing and drying.

3) Species in general are very widely distributed.

4 Subclass Ostracoda - seed shrimps. Two or three pairs of thoracic appendages. Body laterally compressed, and entirely enclosed in a bivalve carapace. Freshwater and marine. Size 0.35-21 mm. No growth lines on valves (cf. Conchostraca). Over 1700 species known, about 1/3 freshwater. "Microscopic clams with legs".

a Widely distributed, clean to polluted water. Generally free living except for a few rare commensals.

b Pollution significance is not known.

5 Subclass Copepoda - copepods. Five - 6 pairs of thoracic appendages, the 1st 4 biramous. Body cylindrical, divided into two sections (Cephalothorax or metasome, and abdomen or urosome). Some parasitic forms are greatly modified. Locomotion by means of 2nd antenna, which is unbranched (cf. Cladocera). Many virtually transparent. Up to 3 mm.

a Distribution world wide, freshwater and marine. One of most abundant of animal plankton.

b Development includes a complex series of growth stages. Eggs carried over from year to year in mud. Resist drying and freezing.

6 Subclass Branchiura - fish lice. With suction cups on head appendages, body strongly depressed, ectoparasitic on fish. Sometimes considered to be an order of the copepoda. Of primary importance as fish parasites.

7 Subclass Malacostraca - (no collective common name). Body usually consisting of 20 segments (approximately 5 in head, 8 in thorax, 7 in abdomen) and 19 pairs of appendages exclusive of eyes. Approximately 30,000 species known nearly 800 in N. America. Of the 12 orders recognized, only 4 have freshwater representatives.

a Order Mysidacea - oppossum shrimps. Essentially a marine group, but three species inhabit our fresh waters. Superficially resemble marine shrimps of commerce. Carapace thin and does not completely cover thorax. Stalked compound eyes extremely large. Nektonic in nature, with thoracic appendages adapted for swimming.

1) Mysis relicta inhabits deep cold oligotropic lakes in northern states east of Great Plains Up to 30 mm Circumboreal.

2) Acanthomysis awatchensis occurs in lakes, rivers, and brackish estuaries of Pacific N W

3) Taphromysis louisianae Gulf coast region, also brackish water Up to 8 mm

b Order Isopoda - aquatic sow bugs or pill bugs Some fifty freshwater species represent approximately 5% of all known species, many of which are terrestrial as well as marine Size 5 - 20 mm

1) Ovoid, flattened dorsoventrally Most of the thoracic and abdominal segments are unfused, giving the animals a many-jointed appearance Lateral extensions of each segment and the absence of any large protruding structures combine to give an overall impression of an army tank in life

2) Generally inhabit springs, brooks and subterranean waters In the north central states they are often abundant in small polluted streams that go dry in summer, of which they are frequently almost the only inhabitants.

c Order Amphipoda - scuds or sideswimmers Chiefly a marine group with about 50 American freshwater species Size 5-20 mm.

1) Body is laterally compressed, few fused segments as in isopods Eyes generally well developed except in subterranean species.

2) Occur in a wide variety of relatively unpolluted waters where ample oxygen is present Generally nocturnal Soft waters generally favored, but Gammarus limnaeus is common in hard waters and Hyalella azteca is sometimes found in alkaline and brackish waters

3) Subterranean species are common in cavernous areas, and hence frequently appear in well waters.

4) Scuds serve as intermediate hosts for a variety of parasites of waterfowl, amphibians, and fishes, but not so far as known for man

d Order Decapoda - freshwater shrimps, crayfish; also marine lobsters, and crabs

Only about 160 species of this huge, essentially marine group, are found in the fresh waters of N. America, of which about 130 are crayfishes. True freshwater crabs occur in Mexico and in the West Indies and one species has been reported in Florida. Other marine crabs occasionally invade fresh waters for extended periods and some have become essentially terrestrial. Decapods are in general from the Rocky Mountain region.

1) Decapod shrimps (prawns) can be distinguished by the laterally compressed rostrum. Commercial freshwater prawn culture techniques have been developed. Size 3-23 cm.

2) The crayfishes (crawdads, crabs) are the predominant group of freshwater decapods. Their shell is heavier and the pincers usually strongly developed. There are many burrowing forms and many species have rather specialized habitat preferences. Unfortunately, however, their pollution significance has not been worked out. In general it can be said that they can tolerate a considerable amount of pollution. Size, (exclusive of antennae): 15-130 mm.

C Class Arachnida - spiders, scorpions, ticks, and mites. The water mites (Parasitegona) have become extensively adapted to fresh waters, and these are almost exclusively restricted to freshwater, there being very few marine and no terrestrial forms. They are readily recognized by their bright colors, globular to ovoid shape, and clambering and swimming habits. Other types of Acari or mites found in marine and freshwater are in the Halacaridae and Oribatei. These crawling types are sometimes found in large numbers in activated sludge. Size 0.4 - 3.0 mm.

1 Superficially resemble minute spiders, but have no division into cephalothorax and abdomen. All evident segmentation has been lost. Four pairs of legs are present in the adult stage.

2 Mites are carnivorous or parasitic, feeding on aquatic invertebrates. Some are commensal on mussels, and host specific.

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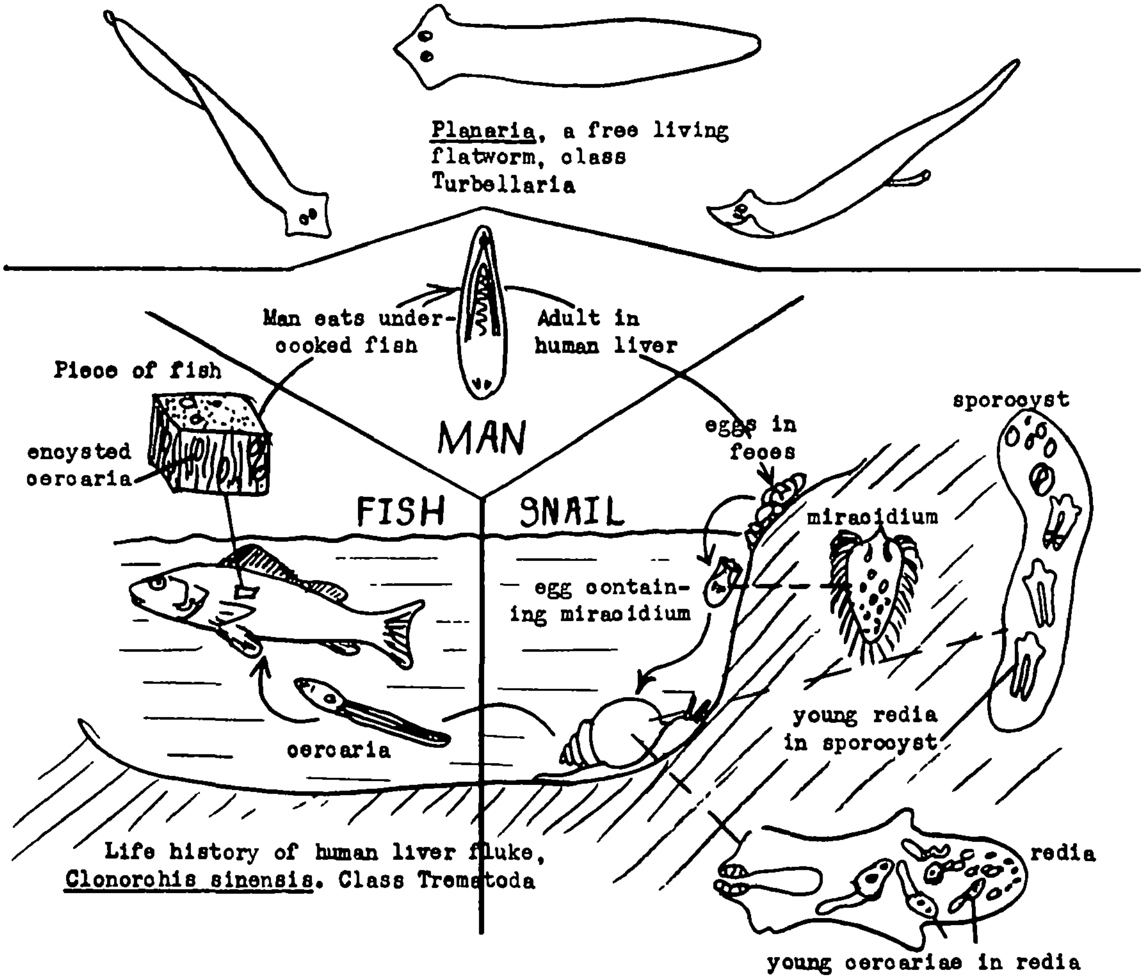
Flatworms

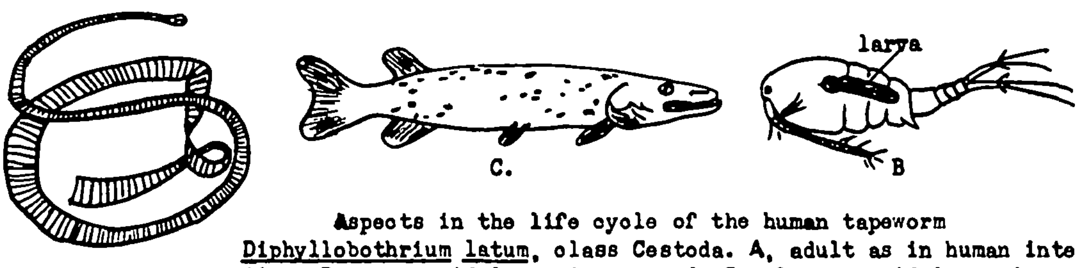
Phylum PLATYHELMINTHES

3/4



Planaria, a free living flatworm, class Turbellaria

Life history of human liver fluke, Clonorchis sinensis. Class Trematoda



Aspects in the life cycle of the human tapeworm Diphyllobothrium latum, class Cestoda. A, adult as in human intestine; B, procercoid larva in copepod; C, plerocercoid larva in flesh of pickerel (X-ray view).

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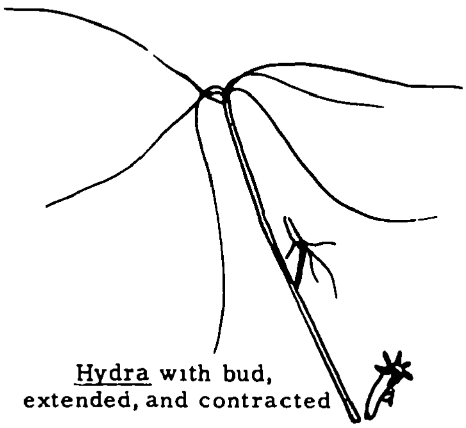
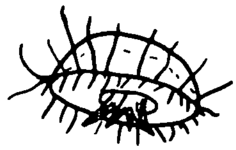
MINOR PHYLA

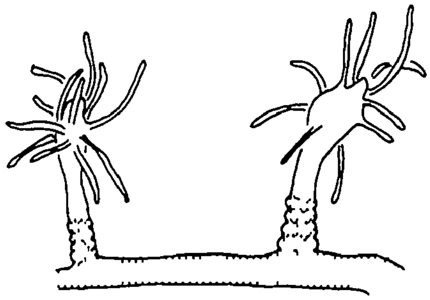
Phylum Coelenterata



Hydra with bud, extended, and contracted

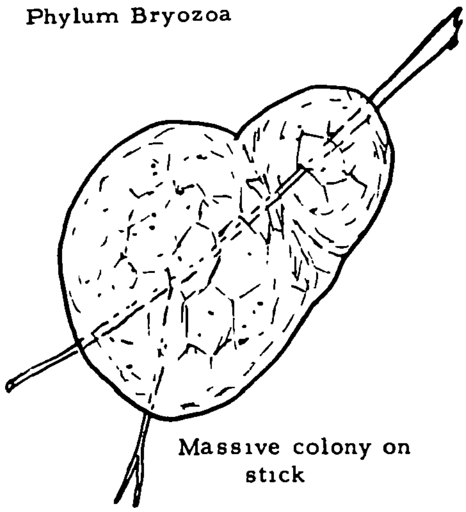


Medusa of Craspedacusta

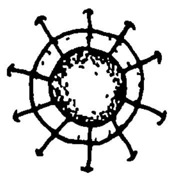


Cordylophora caspia colony

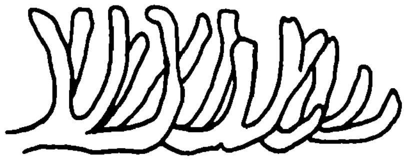
Phylum Bryozoa



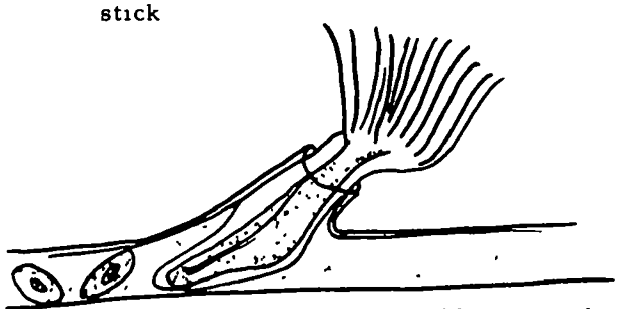
Massive colony on stick



Statoblast



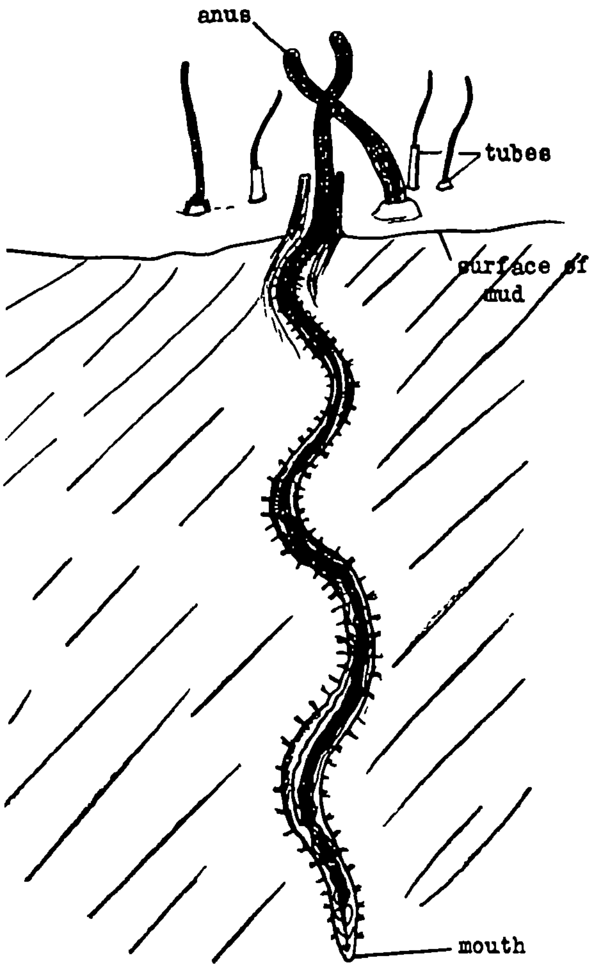
Creeping colony on rock



Single zooid, young statoblasts in tube

FRESH WATER ANNELID WORMS

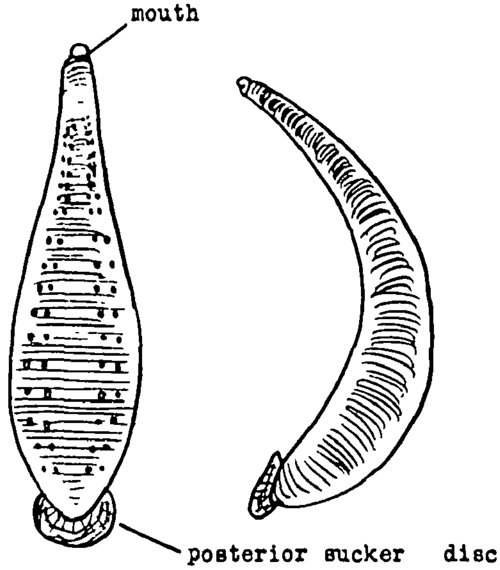
Phylum Annelida



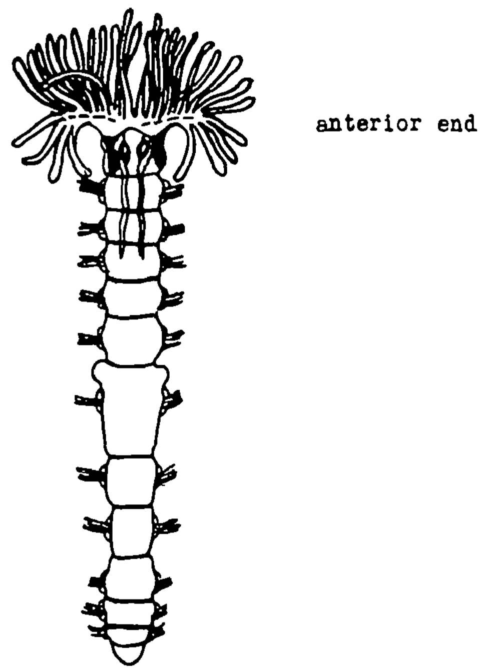
Class Oligochaeta, earthworms

Ex: Tubifex the sludgeworm

(After Liebman)



Class Hirudinea, leeches
(After Hegner)



Class Polychaeta , polychaet worms

Ex: Manayunkia, a minute, rare, tube building worm.
(After Leidy)

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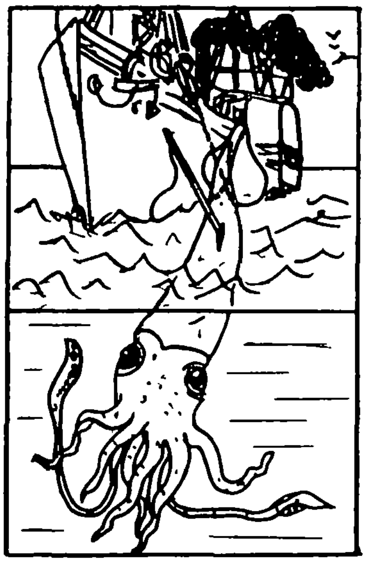
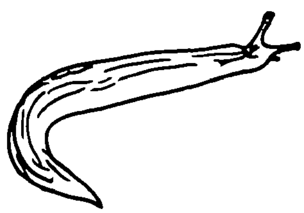
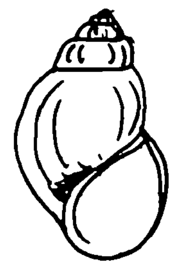
SOME MOLLUSCAN TYPES

Class: Cephalopoda: Squids, octopus, cuttlefish.



Exclusively marine. The giant squid shown was captured in the Atlantic in the early ninteenth century. (After Hegner)





<u>Limax</u>, a slug | **<u>Lymnaea</u>** an air breathing snail | **<u>Campeloma</u>** a water breathing snail

Class: Gastropoda; snails and slugs. (After Buchsbaum)

Class: Pelecypoda; clams, mussels, oysters.

Locomotion of a freshwater clam, showing how foot is extended, the tip expanded, and the animal pulled along to its own anchor. (After Buchsbaum)

PLATE XII d

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Class CRUSTACEA

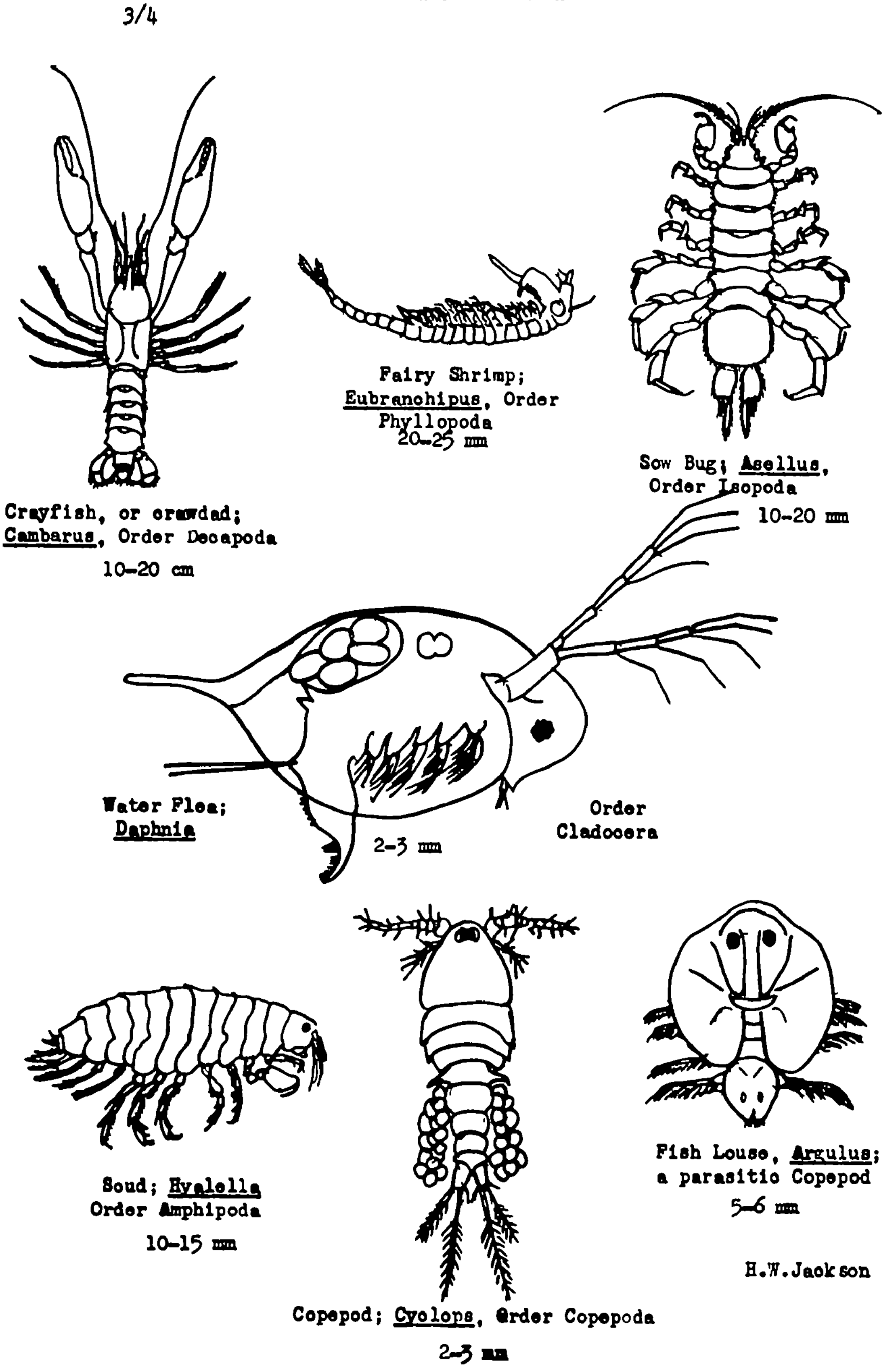

PLATE X

AQUATIC INSECTS

INTRODUCTION

The class Insecta of the phylum Arthropoda includes approximately one million species, more than all the rest of the animal kingdom combined. Nine orders containing some 5000 species inhabit our North American freshwaters. Representatives of these nine orders will be discussed.

Adult insects possess a distinct head, a thorax which bears three pairs of legs and one or two pairs of wings, and an abdomen. The aquatic dwelling immature stages are known as larvae and nymphs. In some orders, the immature stages closely resemble the adults, while in others there is little or no resemblance. Larval insects that resemble the adults mature through a series of "instars" or "molts" called gradual metamorphosis. Those that require changes from egg, larva, pupa to adult undergo complete metamorphosis.

Some insects are able to live in an aquatic environment because their respiratory mechanism is adapted for life in water. Oxygen is distributed to the tissues and CO_2 removed by a system of air-filled tubes called tracheae. Species that have access to the atmosphere through the surface of the water, or which can carry a bubble of air below the surface can "breathe" air directly and are independent of the dissolved oxygen in the water.

Those larvae, pupae, and nymphs that cannot "breathe" free air are sensitive to DO changes. They must purify the tracheal air by exchange with dissolved gases in the water. This is done by means of specialized structures known as tracheal gills and blood-gills. Tracheal gills differ in their shape and position on the animal. Sometimes they are cylindrical filaments on the body, or flat plates on the abdomen.

Also, considerable gaseous exchange is thought to occur through the body's surface. The responses of insects to organic pollution is related to their respiratory needs and capacities.

I ORDER PLECOPTERA (Stoneflies)

Figures 1-2

A The adults are relatively plain, colorless insects of worldwide distribution. They are generally poor fliers Each species is rather definite in the time of its appearance, and many species are most abundant during the late fall or winter.

B The nymphs are all strictly aquatic and relatively similar in structure. They resemble the adults but have "wing pads" instead of wings and two claws on each tarsus. All nymphs have two tail filaments. Filamentous tracheal gills are present in some species, and as a group cannot tolerate low DO's for extended periods.

II ORDER EPHEMEROPTERA (Mayflies)

Figures 3-8

A The adults possess delicate, veined, transparent wings and two long tail filaments. The mouthparts are undeveloped and no food is taken during adult life. Adults sometimes emerge in such numbers near lakes and streams that they constitute a distinct nuisance to man. Emergence usually lasts only one or two days

B The nymphs live in a variety of habitats in streams, ponds and lakes. They have leaf-like tracheal gills attached to the dorsal margins of their abdomens and generally three slender tail filaments posteriorly.

III ORDER TRICHOPTERA (Caddisflies)

Figures 9-16

A The caddisflies are all aquatic. The brown colored adults distinctively fold their wings so as to appear triangular in outline when resting. Generally nocturnal, adult flights may create nuisance conditions. They seldom live over 30 days.

B Larva caddisflies live in protective cases made from debris, sand, or pebbles. Some are portable and others are fixed to stationary structures. Their cases may cover submerged rocks by the thousands.

C Although abdominal filamentous gills are present, their DO requirements cover a wide range and may be quite abundant in organically enriched water.

IV ORDER ODONATA (Dragonflies and Damselflies) Figures 17-21

A Adult dragonflies and damselflies are large conspicuous foragers of almost any body of water. They can be seen gracefully, flying just above the water's surface in search of other insects. They hold their wings horizontal even when resting.

B Odonate nymphs have a distinctive food-grabbing device located under their mouth used to capture passing prey. Some lie concealed, others crawl about.

1 Dragonfly nymphs have unique enlargement of hind intestine, lined with rows of tracheal gills that are not visible externally. Water is kept moving by "breathing" pulsations of abdomen and the expelled water can aid in propulsion.

C Damselfly larvae on the other hand have three leaf-like external tracheal gills located on the rear of the abdomen. These are vertical to the body and used to propel the nymph.

D The ability of these nymphs to migrate rapidly from one location to another makes their value as pollution indicators doubtful.

V ORDER HEMIPTERA (True Bugs) Figures 22-26

A Hemiptera are readily distinguished by the combination of piercing and sucking mouthparts, the anterior wings are leathery at the base and membranous apically while the hind wings are entirely membranous. Most hemipterans are terrestrial. Of the aquatic members, many live on or near the water's surface. The change-over from larva to adult is gradual.

B No tracheal gills are known in the order. Air is carried beneath the surface under the wings or in masses of fine hairs. Some Hemiptera, such as water boatmen, are commonly found in polluted water. Surface dwellers, such as water striders are quickly immobilized by a film of oil.

VI ORDER MEGALOPTERA (Dobsonflies, Alderflies, Hellgrammites) Figures 27-29

A The adults are dull colored, up to 70 mm in length. Although widely distributed, they are seldom found in large numbers and little is known about their life history.

B The larvae are among the most striking of aquatic insects. The body is stout, elongated, and up to 90 mm in length. A pair of lateral, cylindrical, fleshy gill filaments are located on each abdominal segment. The legs are well developed and the jaws strong and conspicuous.

VII ORDER LEPIDOPTERA (Aquatic Moths)

A Only members of a single family (out of 150) are known to have aquatic larvae. The adults are small dull colored moths.

B The larvae are widely distributed over U.S. in ponds with heavy growth of water-lilies and similar vegetation. Their bodies are whitish except for the dark head capsules which bear strong jaws. Branching filamentous gills are present on the abdomen. The legs are reduced to fleshy protuberances (prolegs).

VIII ORDER COLEOPTERA (Beetles) Figures 30-34

A The adult beetle form is well known. Horny or leathery covers represent the front of two pairs of wings. Hind wings are membranous and folded underneath. Mouthparts are for biting. Some adults are not structurally modified for aquatic life (riffle beetles) and crawl about on the stream bottom. In others the hind legs may be greatly modified for swimming. Most are dependent on atmospheric oxygen that must be periodically replenished at the surface.

B Some larvae are predacious and others herbivorous. The head, thorax, and abdomen are usually distinct and chitinized. They respire by means of tracheae, lateral gills, or anal gill tufts. The group, as a whole, has a wide range to pollution.

IX ORDER DIPTERA (True Flies, Mosquitoes, and Midges) Figures 35-39

A The adults are small delicate insects and may be observed in swarms near a body of water. They have one pair of functional wings and a vestigial pair (reduced in size).

B The larvae are found in a variety of aquatic habitats, and may attain enormous numbers if tolerant to organic pollution. Although the larvae are typically cylindrical and wormlike, there are many morphological adaptations for different modes of life. The head may or may not be well developed. Some members of the Chironomidae (Midges) have an accessory respiratory mechanism erythrocruorin (haemoglobin) in the blood to permit life in low DO's. These "bloodworms" also may possess cylindrical blood-gills on the eleventh body segment.

C Pollution tolerance in the Diptera has been associated with certain species. The group is being used increasingly by biologists to evaluate the effects of pollution on aquatic life.

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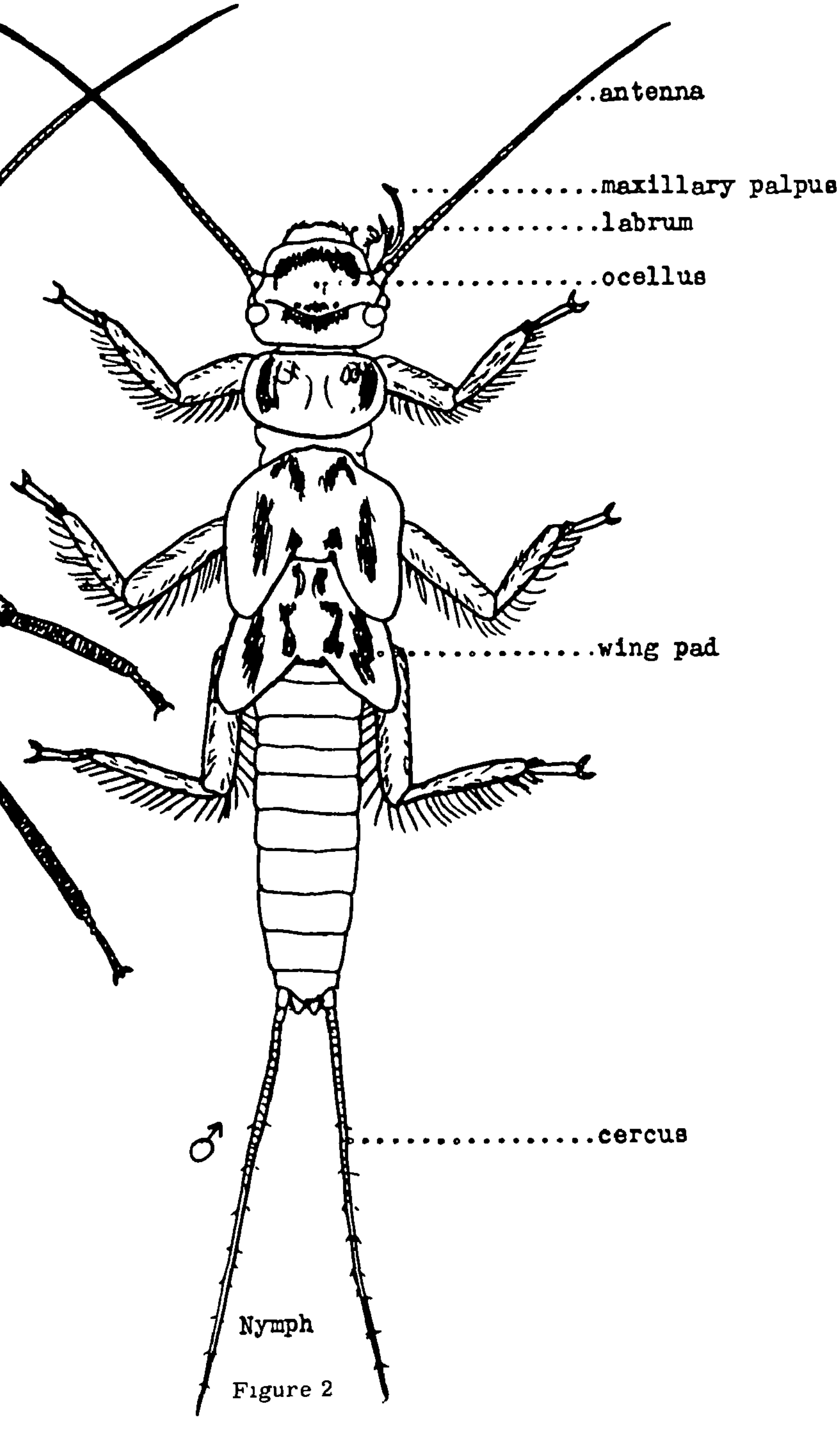
This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, and revised by W.T. Mason, Jr., Aquatic Biologist, Analytical Quality Control Laboratory, WPO, EPA.

Stoneflies
Order PLECOPTERA
(Isoperla confusa)

Adult

Figure 1

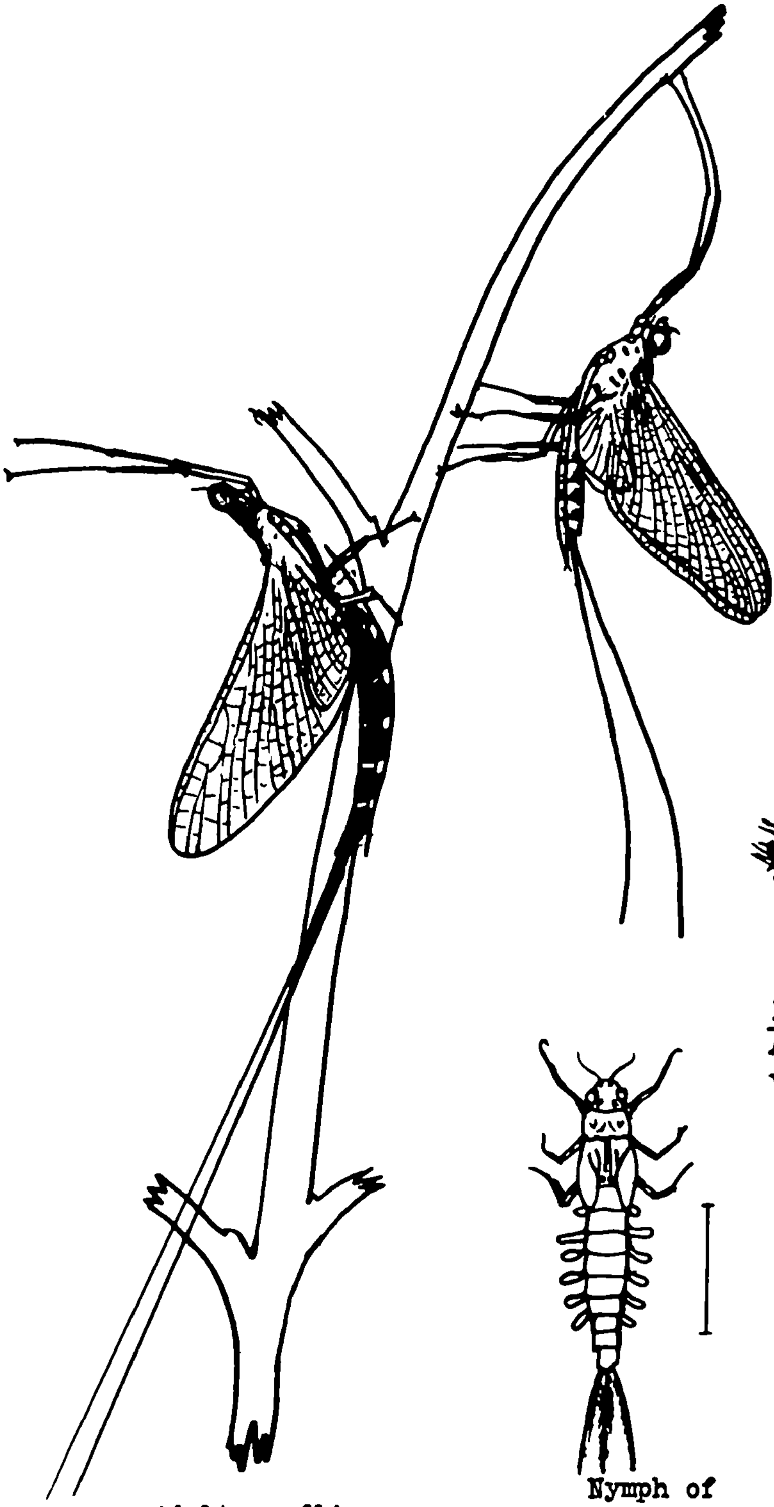


Nymph

Figure 2

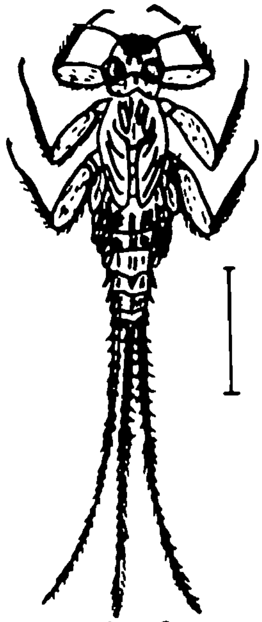
HWJ

May Flies
Order EPHEMEROPTERA



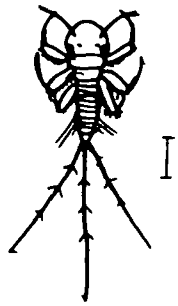
Adult mayflies (Heptageniidae)

Figure 3



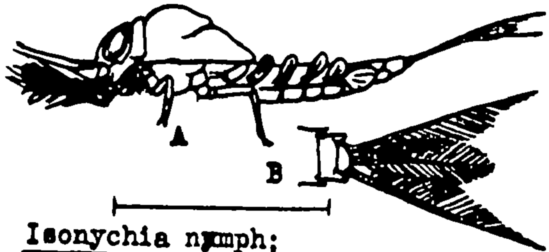
Nymph of Stenonema

Figure 4



Young nymph of Stenonema

Figure 5

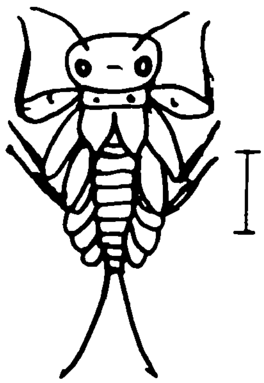


Isonychia nymph; A, side view; B, dorsal view of tail.

Figure 7

Nymph of Baetis

Figure 6

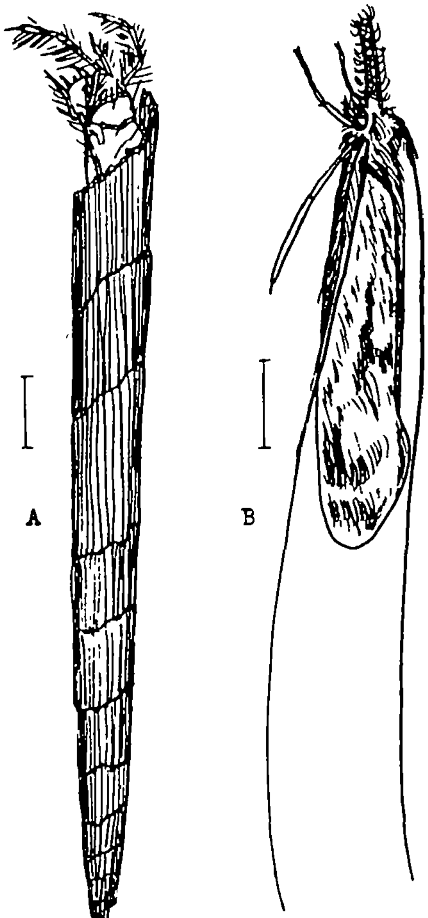


Iron nymph NOTE: Only two tail filaments

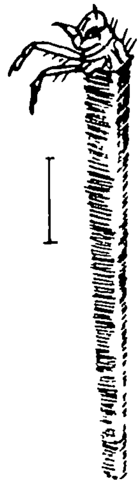
Figure 8

HWJ

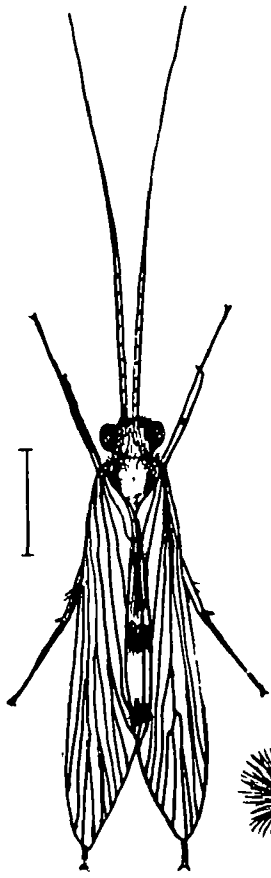
Caddis Flies
Order TRICHOPTERA

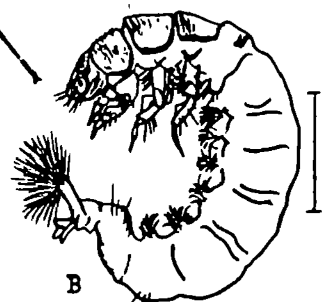


Triaenodes
A larva and case, B.adult.
Figure 9

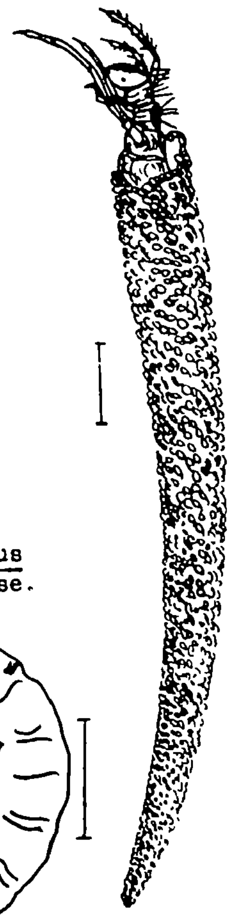


Brachycentrous
Larva and case.
Figure 10

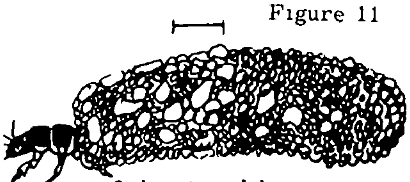




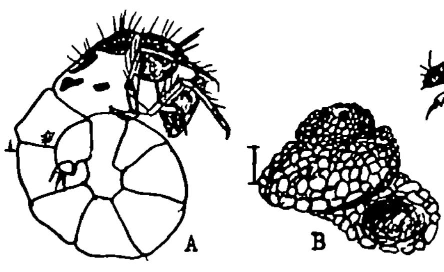
Hydropsyche
A adult; B.lavra.
Figure 11



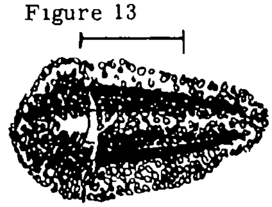
Leptocella
Larva and case.
Figure 12



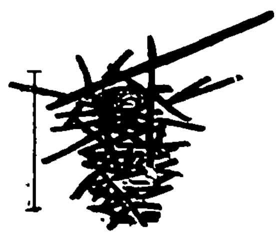
Ochrotrichia
Larva and case.
Figure 13



Helicopsyche
A larva; B.case.
Figure 14



Molanna larva in case
Figure 15

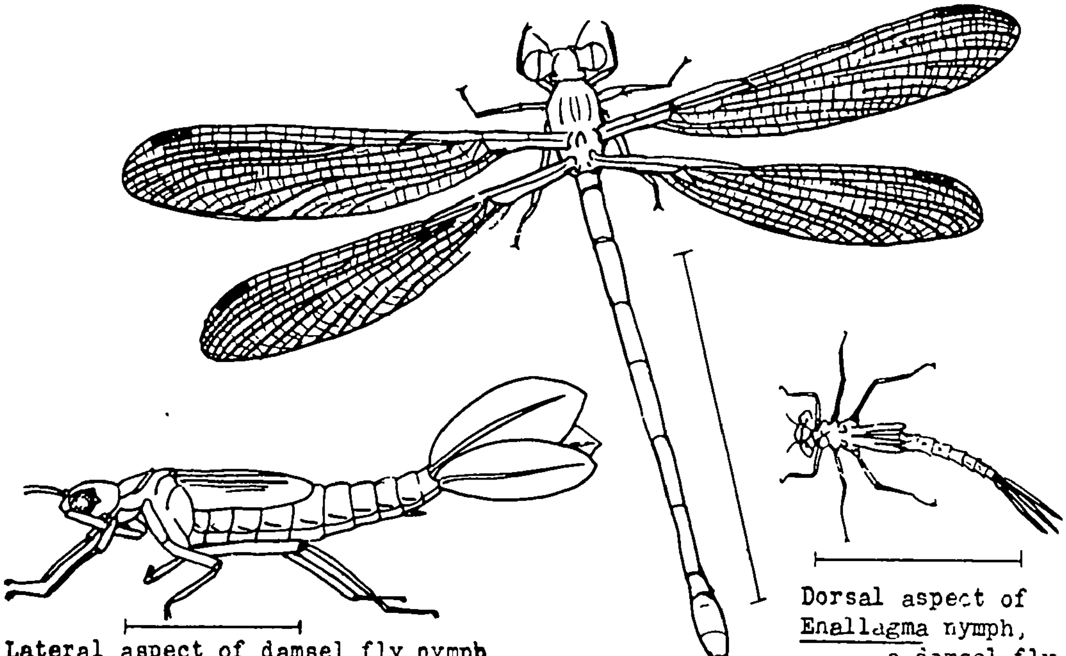


"Log cabin" type case of Limnophilid larva.
Figure 16

H.W.J

Dragon Flies and Damsel Flies

Order ODONATA



Typical adult damsel fly

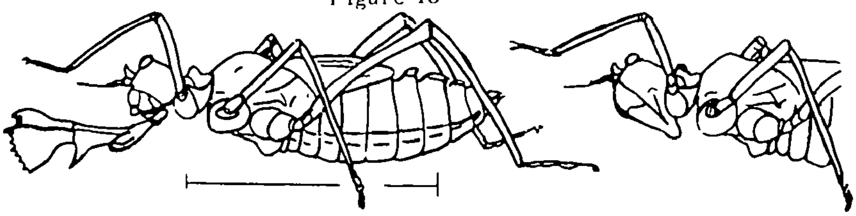
Figure 18

Lateral aspect of damsel fly nymph Argia showing caudal tracheal gills. Note labium folded under head.

Figure 17

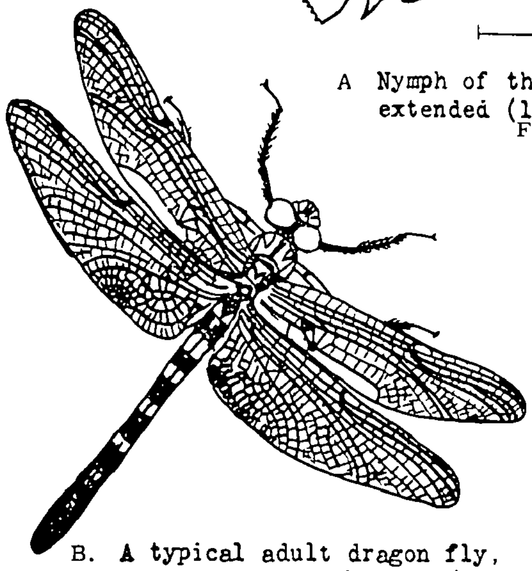
Dorsal aspect of Enallagma nymph, a damsel fly.

Figure 19



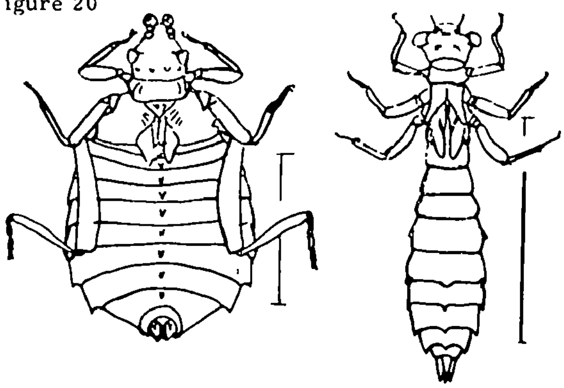
A Nymph of the dragon fly Macromia showing labium extended (left) and retracted.

Figure 20



B. A typical adult dragon fly, Macromia. (2-3 in.)

Figure 20

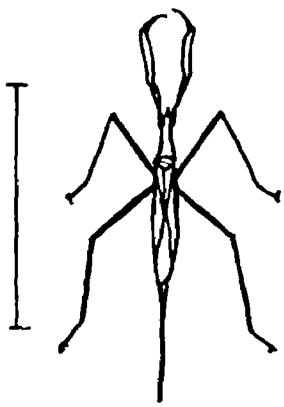


Dragon fly nymphs Hagenius (left) and Nasiaeschna.

Figure 21

HWJ

True Bugs, or Sucking Insects
Order HEMIPTERA



Water Scorpion
(Ranatra spp.) Crawls about in shallow water and elevates "snorkel" tail to surface to breath air.

Figure 22

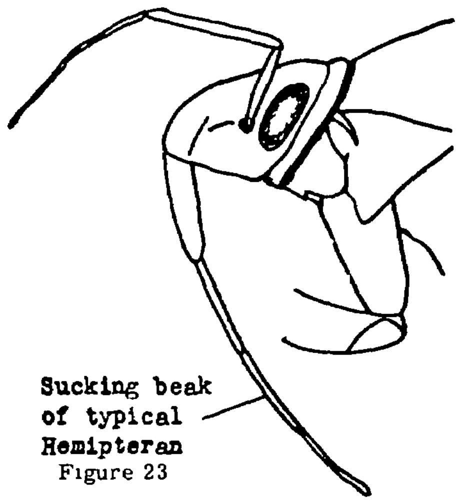
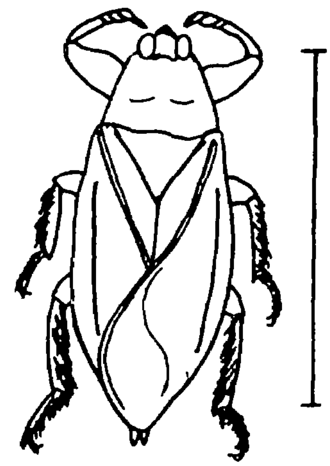


Figure 23

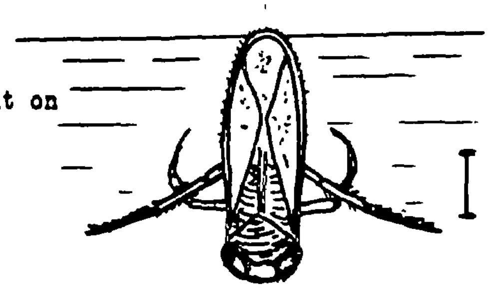


Electric-light bug
(Lethocercus sp)
Extremely predacious on small aquatic organisms. An active flier positively phototropic.

Figure 24

Water Strider
(Gerris spp.)
Jumps and glides about on the surface film.

Figure 25

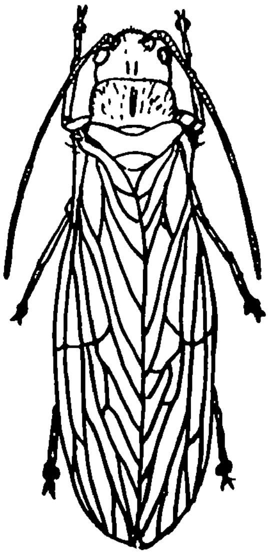


Water Boatmen
(Corixa sp.)
This individual is shown with the abdomen just piercing the surface film to renew his air supply. In well aerated water they are able to remain submerged almost indefinitely.

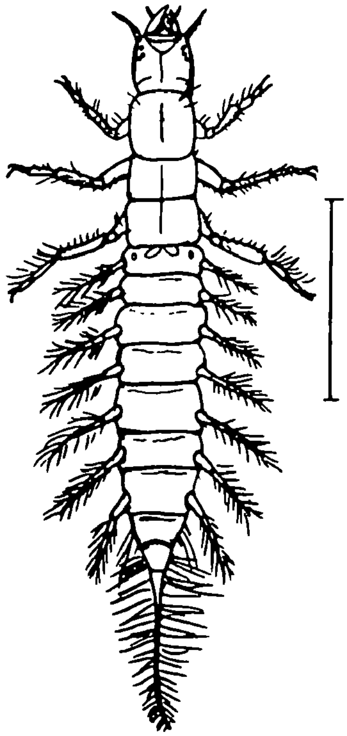
Figure 26

HWJ

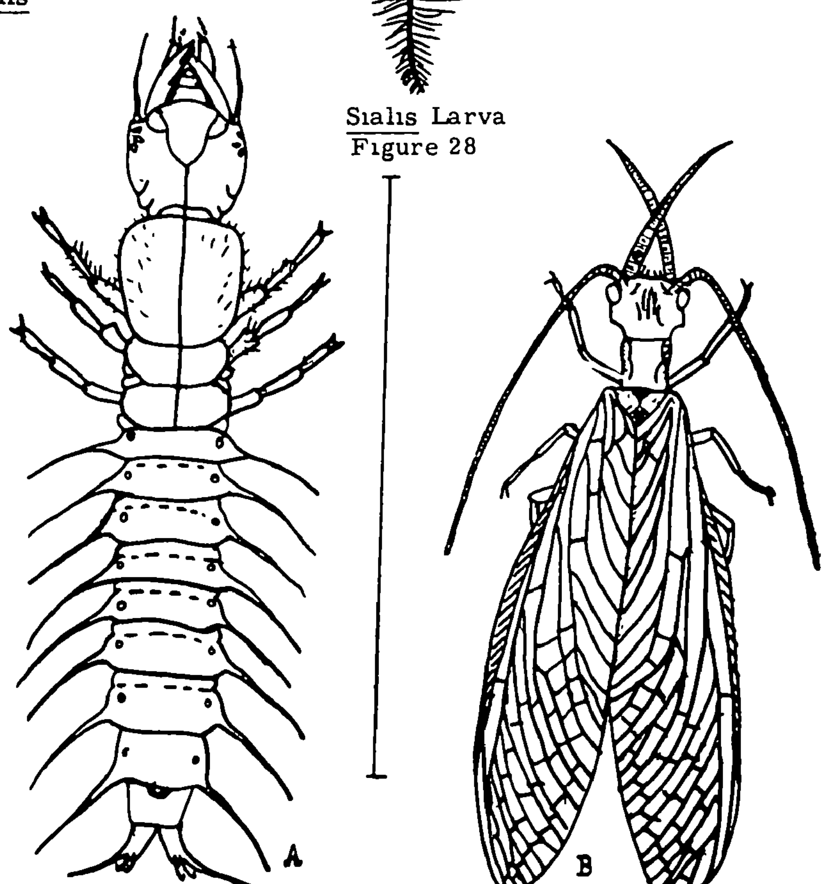
Alderflies, Dobsonflies, and
Hellgrammites
Order MEGALOPTERA (Neuroptera)



Alderfly Sialis
Adult
Figure 27



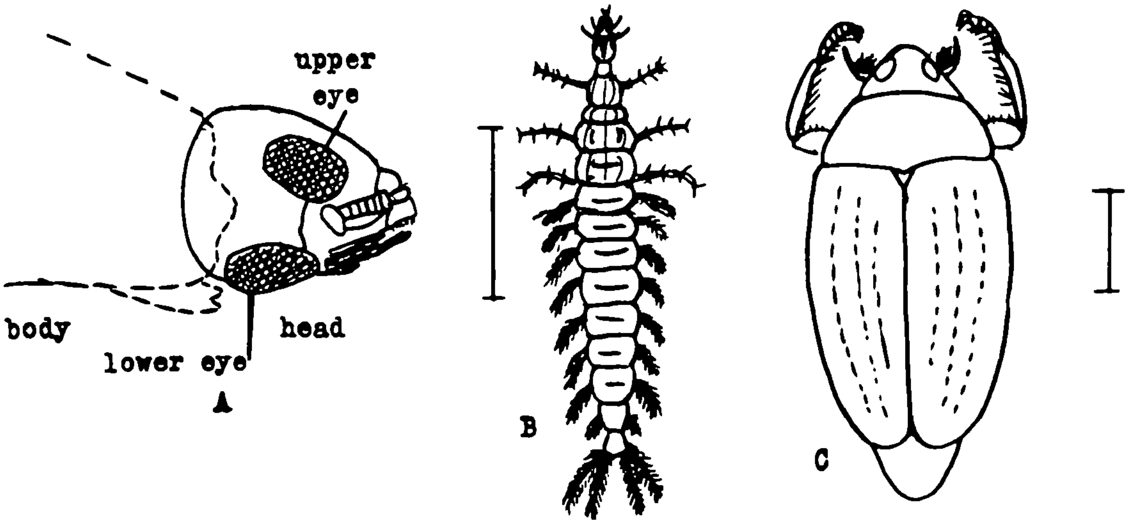
Sialis Larva
Figure 28



HWJ

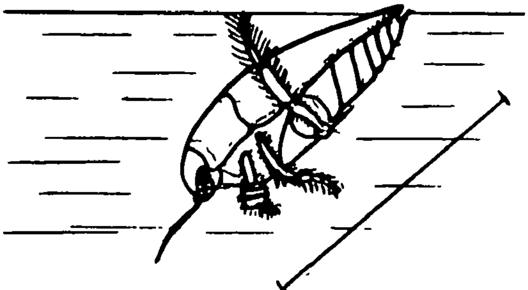
Figure 29 Dobsonfly Corydalus cornutus
A, larva (hellgrammite), B, adult

Beetles
Order COLEOPTERA



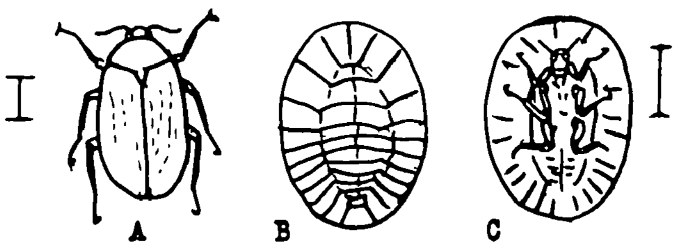
Whirligig beetle (Gyrinus) A, Side view of head of adult showing divided eye; B, Larva; C, Adult. Carnivorous.

Figure 30



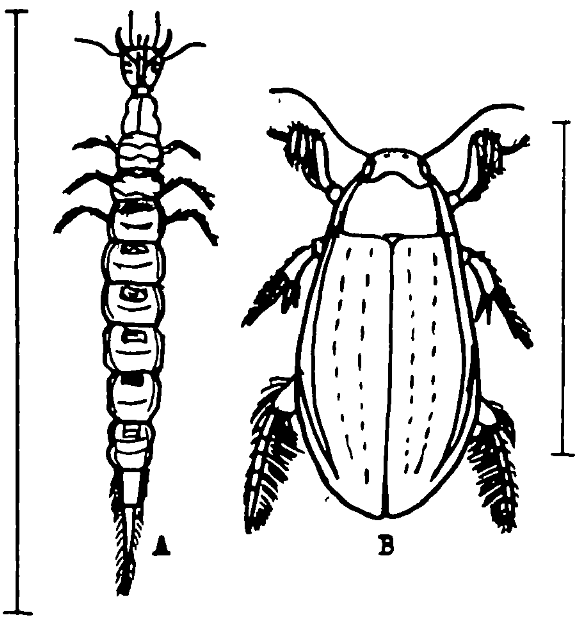
A diving beetle (Dytiscus) taking air at the surface.

Figure 31



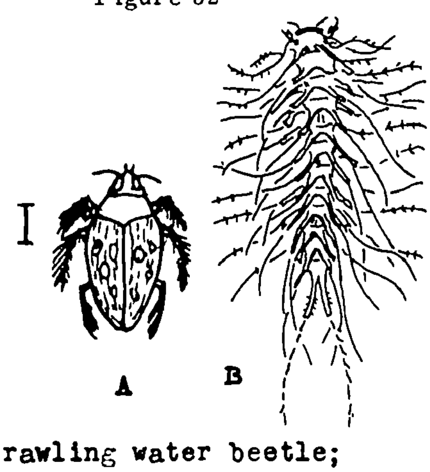
The Waterpenny (Psephenus); A, adult; B, dorsal side of larva; C, ventral side of larva. Predominantly herbivorous.

Figure 32



A diving beetle (Cybister). The diving beetles include some of the largest and most vorasious of aquatic insects. A, larva; B, adult.

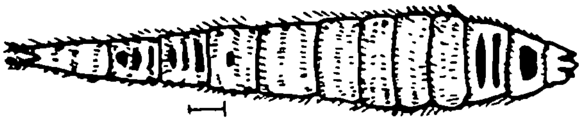
Figure 33



Crawling water beetle; A; adult; B, larva. Predominantly herbivorous.

Figure 34

True Flies
Order DIPTERA

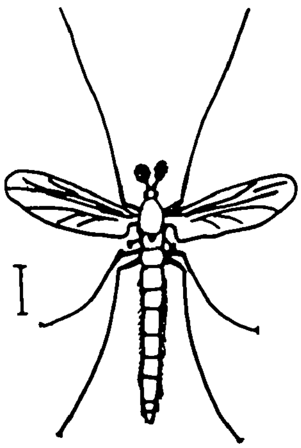


A. Sewage fly larva
(Psychoda)

B Sewage fly pupa
(Psychoda)

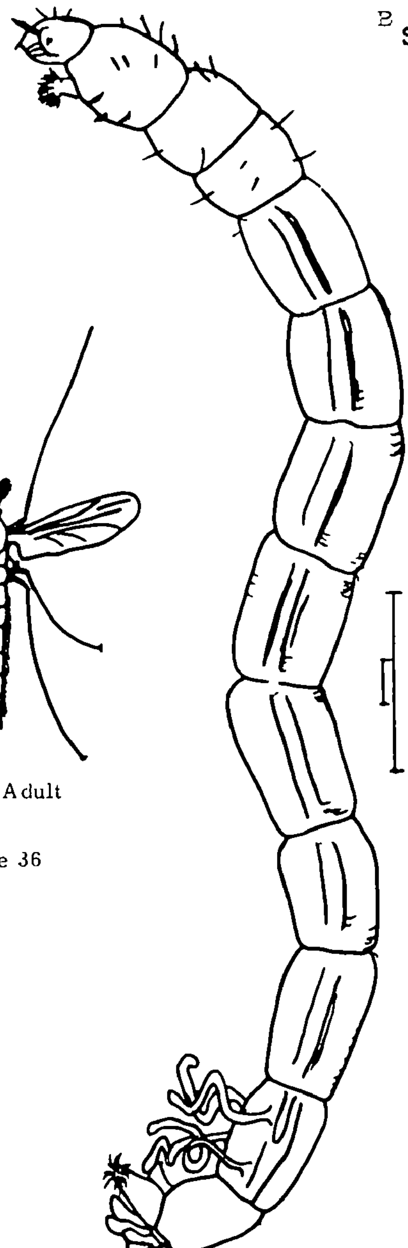
Midge pupa
(Chironomus)

Figure 35



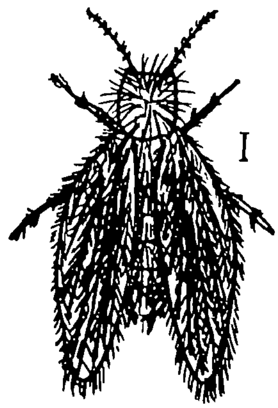
Midge Adult

Figure 36



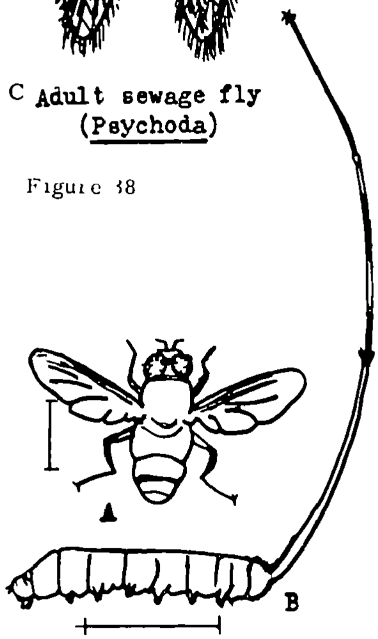
Midge larva
(Chironomus)

Figure 37



C Adult sewage fly
(Psychoda)

Figure 38



Rat-tailed maggot
(Eristalis)
A, adult; B, larva.
Figure 39

HWJ

KEY TO ORDERS OF AQUATIC INSECTS

1	Without jointed thoracic legs; maggot-like... Larvae of DIPTERA (flies) Figure 37	
1'	Jointed thoracic legs present Figure 2	2
2	Long, segmented appendages at posterior end Figure 2 and 4	3
2'	Posterior appendages absent, or if present, not long and segmented Figure 28 and 33 A	4
3	Two posterior appendages, two tarsal claws, usually with finger-like tracheal gills on ventral side of thorax. Nymphs of PLECOPTERA (stoneflies) Figure 2	
3'	Usually three (sometimes two) posterior appendages; one tarsal claw, tracheal gills on lateral margins of abdominal segments. Nymphs of EPHEMEROPTERA (mayflies) Figure 4	
4	Wings or wing pads present, nymphs and adults Figure 24	5
4'	Without wing pads; larvae Figure 28	8
5	Wings, hard or chitinous (elytra), hind wing may be complete or vestigial. COLEOPTERA (beetle adults) Figure 33B	
5'	Wing pads present, nymphs Figure 21 and 26	6
6	A long jointed beak present. Nymphs and adults of HEMIPTERA (bugs) Figure 23	
6'	Without a long jointed beak; labium, when extended, long and scooplike, and when folded, serving as a mask covering the other mouth parts. Nymphs of ODONATA Figure 20	7
7	Platelike caudal gills present; damselflies. Figure 17	
7'	No platelike caudal gills; dragonflies. Figure 21	
8	Five pairs of abdominal prolegs. LEPIDOPTERA (aquatic caterpillars)	
8'	Prolegs absent, or confined to last abdominal segment	9
9	Each abdominal segment with one pair of stout lateral processes MEGALOPTERA (hellgrammites, alderfly and fishfly larvae) Figures 28 and 29A	
9'	No stout lateral abdominal processes; sometimes with long, thin lateral filamentous processes (a few beetle larvae have four stout hornlike processes on each body segment) Figure 30B	10
10	A pair of terminal abdominal prolegs, usually in fixed or portable cases. TRICHOPTERA (caddis fly larvae) Figure 11B	
10'	Without terminal prolegs. COLEOPTERA (beetle larvae) Figure 30B	

This outline was prepared by John E. Matthews, Aquatic Biologist, Robert S Kerr Water Research Center, Ada, Oklahoma 74820.

FRESHWATER CRUSTACEA

Part 1. Crustacean Diversity

I The two major classes of arthropods in freshwater are crustacea and insects. The diagnosis of the class Crustacea is simply, "arthropods with two pairs of antennae."

A Crustacea share these features:

1 The great majority of the 30, 000 known species of crustaceans are marine. Only a very few are terrestrial.

2 Two pairs of antennae. The first pair are antennules.

3 Three pairs of masticating and feeding appendages.

4 The paired appendages are typically biramous (Figure 1)

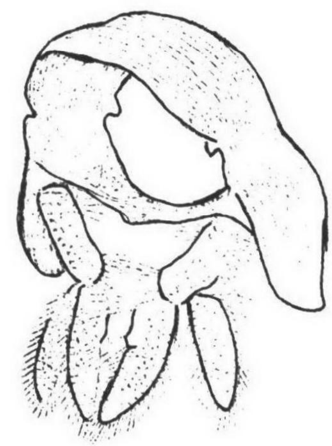


Figure 1. Abdominal Ring of a Decapod (from Schmitt)

TABLE 1

CLASSIFICATION

CLASS CRUSTACEA	
("Microcrustacea" - Entomostraca)	
Subclass Branchiopoda	Subclass Malacostraca
Order Anostraca	Division Pericarida
Order Notostraca	Order Mysidacea
Order Conchostraca	Order Isopoda
Order Cladocera	Order Amphipoda
Subclass Ostracoda	Division Eucarida
Subclass Copepoda	Order Decapoda
Order Calanoida	
Order Harpacticoida	
Order Cyclopoida	

B Classification into two groups is admittedly artificial.

1 Entomostraca, an old classification, is used now as a general descriptive term for the generally smaller sized crustacea in eight diverse orders; often called microcrustacea.

2 Malacostraca is a natural group, all members possessing nineteen segments (plus one embryonic)(Figure 2).

II CRUSTACEA AND MAN

A Crustacea are important as first order consumers. The Cladocerans and Mysids are essential in the food chain, a link between the algae and many fish species.

B Commercial fisheries exist for crayfish in Louisiana.

C The Sins of Crustacea

1 One group of copepods is parasitic upon fish.

2 Amphipods are destructive to fish nets in some lakes.

3 Case making amphipods seriously contribute to the irrigation canal wall growths which reduce flow.

4 Some copepods are intermediate hosts for vertebrate helminth parasites.

5 Microcrustacea have been the cause of spotting of paper in paper mills. Oligotrophic lakes serving as a water supply for the mill contained sufficient numbers of copepods to seriously spot paper rolls. The pigment, astaxanthin, within the cells of the copepod turns red, leaving discolored spots in the finished paper.

III Crustacean life history involves distinct stages from egg to adult.

A The first larval stage is the nauplius, plural nauplii (Figure 3).

1 The malacostra brood the eggs, often in marsupia, thus naupliar stages are passed in the egg membrane.

2 The "entomostraca" generally have free swimming nauplii.

a Cladocera, with the exception of only a few genera, do not have a free-swimming nauplius stage.

b The great majority of nauplii encountered in freshwater zooplankton are copepods.

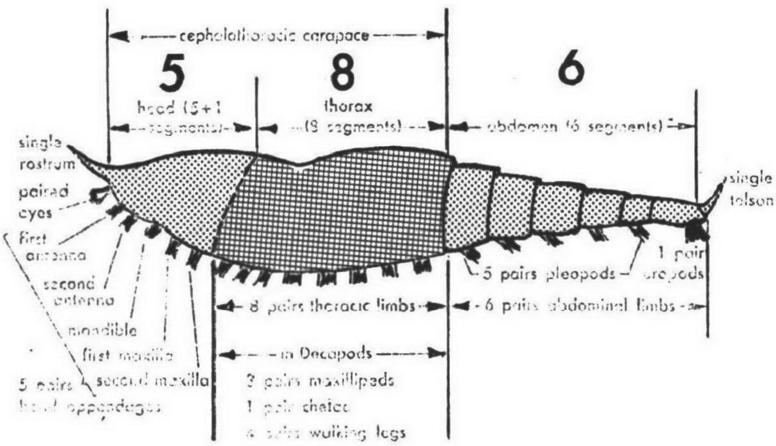


Figure 2. Malacostracan Organization (after Russell-Hunter)

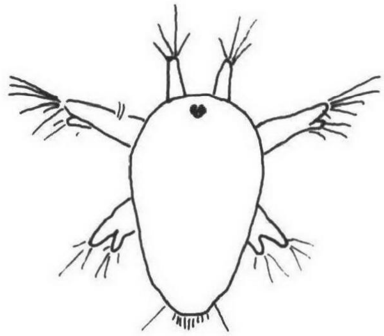


Figure 3. Nauplius Larva

FRESHWATER CRUSTACEA

Part 2. Entomostraca

I As implied, the Anostraca are without a shell or carapace

A They are the least specialized of the Branchiopods.

1 Trunk limbs are all similar and used both in swimming and feeding

2 They are filter feeders.

3 Types include

a Artemia, the brine shrimp

b Branchipus, living in brackish water

c Chirocephalus and other genera, known as fairy shrimp, living in ponds which dry up in the summer.

4 Size is generally up to two cm, but some reach as much as ten cm as adults

B Artemia, the brine shrimp, is commercially valuable.

1 The adults are sold frozen in bulk.

2 The dried eggs which can be stored in diapause and hatched as needed are widely used in fish culture.

II Notostraca or tadpole shrimp have a carapace forming a dorsal shield which covers only about half of the body.

A Only two genera are known, Triops and Lepidurus.

B Triops has been a serious pest of rice fields as it stirs up the bottom sediments, killing the rice seedlings.

III The Conchostraca possess a bivalve shell, completely enclosing body and limbs, resembling the shell of a small clam.

IV Cladocera have a similar bivalve shell or carapace except the head is left free The second antennae are the main means of a jumpy movement, hence the name water fleas

V Ostracoda have a bivalve carapace and resemble Conchostraca except for the absence of growth lines on the valves.

A Average size is 1 to 3 mm.

B They are found in all types of fresh water

C Large populations may develop in algal cultures and aquaria. This habit is detrimental to culture of aquatic snails.

VI The Copepoda, like the Cladocera, are almost universally distributed in the plankton, benthic, and littoral regions of fresh water

A Copepods in the Order Harpacticoida are substrate dwellers, the other two orders Cyclopoida and Calanoida are mainly swimmers (Figure 4).

B Copepod eggs hatch into a small larva, the nauplius, which has three pairs of stubby bifurcate appendages, representing the first antennae, second antennae, and mandibles (see Figure 3).

C As in the Cladocera some common species of planktonic copepods exhibit diel verticle migrations in lakes and ponds, with a greater concentration of individuals in the upper waters during the hours of darkness

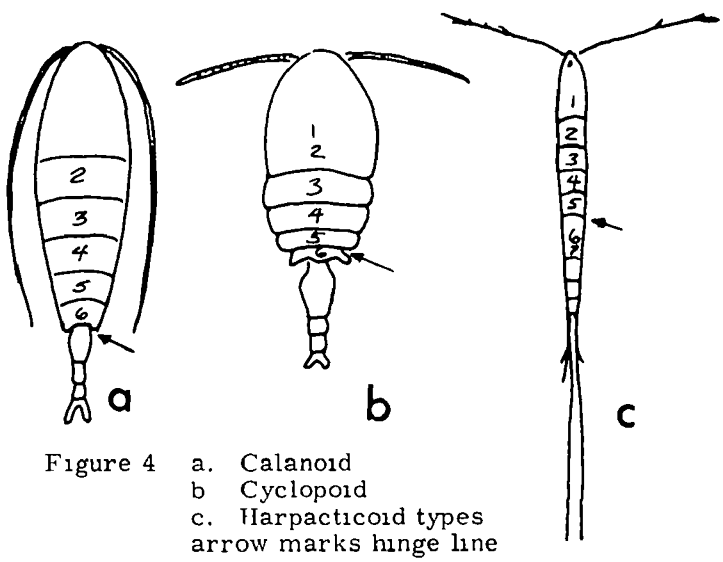


Figure 4 a. Calanoid
b Cyclopoid
c. Harpacticoid types
arrow marks hinge line

FRESHWATER CRUSTACEA

Part 3. Malacostraca

I The subclass Malacostraca includes four orders of crustacea, and contains almost three quarters of all the known species of crustacea.

A Characteristics of this group distinguishing them from the six orders commonly termed "Entomostraca" include·

1 Tagma (an organized group of segments or somites forming a distinct body section) 5 - 8 - 6.

2 A gastric mill in the stomach.

3 The larva is usually passed within the egg, and when present, it does not feed.

B The most primitive of this group are filter feeders.

II ORDER MYSIDACEAE

A The mysids or opossum shrimp occur in the marine plankton and over littoral sand-flats; the few forms in fresh waters are relatively recent marine relict species as shown by physiological investigations.

B The last pair of pleopods are enlarged, containing statocysts, being used as rudders and elevators for the swiftly darting mysids.

C Three freshwater species occupy different areas of North America.

1 Acanthomysis awatchensis is found in streams on the Pacific Coast.

2 Mysis relicta is found in the Great Lakes and northward.

3 Taphromysis louisianae is found in streams of the Gulf Coast (Figure 5).

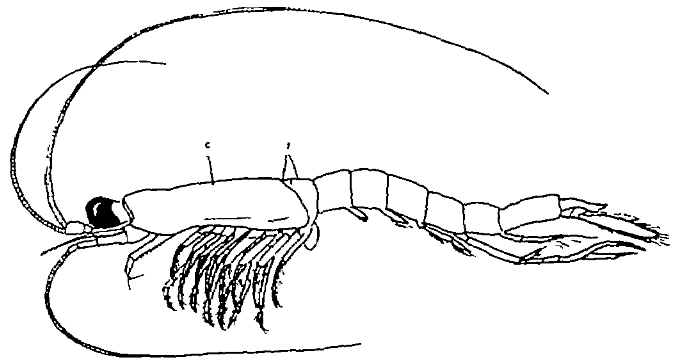


Figure 5. Mysidacea. Lateral view of Taphromysis louisianae (c, carapace; t, free thoracic somites). Modified from Banner.

III ORDER ISOPODA

A The most striking characteristic of the isopods is the dorsoventrally flattened body (Figure 6) which is in contrast to the lateral compression of most amphipods. Thus most isopods crawl at least part of the time. Lygia, a marine isopod, can move each leg sixteen steps per second. Other isopods can run backward as rapidly as forward.

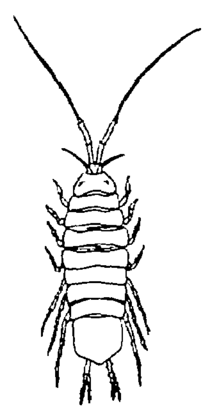


Figure 6

B The most common freshwater isopods (log lice, sow bugs, water slaters) in North America belong to the genera Lirceus and Asellus (Order Asellota, Family Asellidae).

1 These isopods typically form the well known third peak below organic discharges, e.g., Tubificidae, Chironomidae, Asellidae.

2 Lirceus. The lateral margins of the head are produced to overhang the base of the mandibles, the anterior edge of head is produced (Figure 7).

Figure 7

Lirceus

3 Asellus. The above characters negative.

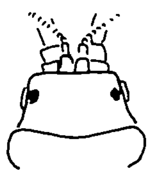


Figure 8

Asellus

IV ORDER AMPHIPODA (Figure 9)

A Another difference between isopods and amphipods, also concise and consistent is in the thoracic and abdominal appendages, each being arranged in at least two groups. In the isopods all thoracic limbs and all abdominal limbs are similar.

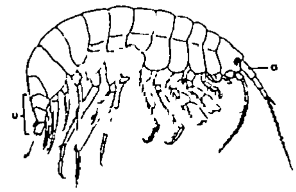


Figure 9 Amphipoda. Lateral view of Gammarus a, first antenna, u, urosome

B Most freshwater amphipods are substrate oriented and active in swimming.

1 Pontoporeia is both benthic and nektonic, a glacial relict of both Eurasia and North America

2 Corophium spinicorne, a tube building amphipod, is found on the West Coast. It is a major contributor to the community lining irrigation canal walls.

V ORDER DECAPODA

Includes freshwater shrimp, crayfish and crabs.

A Decapods are distinguished from all other Malacostracans in that the first three pairs of thoracic appendages are modified as maxillipeds. The remaining five pairs of thoracic appendages are legs, hence, the name decapoda. The legs are chelate, the first pair which is often heavier is called a cheliped

B Two suborders are recognized.

1 Natantia (natant - "swimming"), the body generally adapted for swimming and laterally compressed. The rostrum is keeled(Figure 10). This suborder includes the freshwater shrimp.

2 Reptantia (reptant - "crawling"), the body generally adapted for crawling and dorso-ventrally flattened to a degree. The rostrum is flattened dorso-ventrally (Figure 11). This suborder includes crayfish and only a few freshwater crabs.

Figure 10

Figure 11

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FRESHWATER MOLLUSCA

Part 1. General Concepts

I In terms of number of individuals, number of species, and energy flow through the group, molluscs are clearly a major phyla ranking only below the Arthropoda in this respect.

A The term mollusc means soft, referring to a soft body within a hard calcareous shell.

B Extensive use is made of cilia and mucous mechanisms, in feeding, locomotion, respiration, and reproduction.

C The hard parts of calcareous shell have long been used in systematics in this group.

1 The gastropods or univalves have a single shell spirally coiled in one or more planes, or have a low cone shaped shell, as in the limpets. In both these groups the body is spirally coiled and asymetrical.

2 The bivalves have two calcareous valves united by an elastic hinge ligament. The body is bilaterally symetrical.

II Structure and function are homologous in both gastropods and bivalves with some exceptions.

A The soft body is characteristic. Prominent features are·

1 The foot is used for locomotion - gliding in many gastropods and burrowing in bivalves and some gastropods. As in other soft parts, the foot is a remarkable hydraulic skeleton or haemoskeleton which allows for startling changes in shape.

2 Gills or ctenidia. This feature is characteristic of the phylum mollusca only.

B The shell is composed primarily of calcium carbonate and thus, reflects water chemistry.

C Reproduction

Typical stages following the egg are the trochophore, veliger, pediveliger, and juvenile. This basic plan is followed in both orders, whether oviparous, larviparus, or viviparus.

D Feeding is typically filter or suspension feeding and browsing.

1 Gastropods possess a radula. In the molluscs this is lacking only in the bivalves. The radula is used for rasping food from the substrate or detritus. Some gastropods may also suspension feed.

2 Bivalves are suspension or filter feeders. They possess a crystaline style (some gastropods also have this) which revolves in a style sac taking the food chain (a rope of mucous with food particles) sorted by the palps, in a windlass type operation down the food canal. This is the only revolving organ in nature. As it rotates against a gastric shield, enzymes are liberated for food digestion.

Synoptic Classification - Phylum Mollusca	
Class Gastropoda	
1	Order Mesogastropoda
2	Order Basommatophora
Class Bivalvia	
3	Order Schizodonta
4	Order Heterodonta

FRESHWATER MOLLUSCA

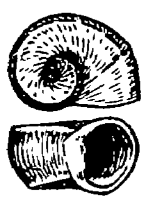
Part 2. Gastropoda (Snails and Limpets)

I The Class Gastropoda is represented in virtually every aquatic habitat from cave streams to swamps. All are substrate oriented, and are found in lakes at depths as great as 250 meters to shallow pools. Each body of water has its characteristic snail fauna. Although some groups, for example, are referred to commonly as River Snails (family Pleuroceridae) and Pond Snails (Lymnaeidae), they are not so restricted.

II GENERAL CHARACTERS

The majority have a spiral (cone or discoidal) The limpets have a shell shaped like a coolie hat.

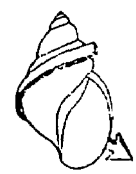

1 Spiral

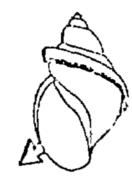

2 discoidal


3 limpet

A The shell is composed of calcium carbonate and covered by an organic layer, the periostracum.

1 If the shell aperture is on the observers right (coiled counter clockwise), it is dextral and if to the left (coiled clockwise), it is sinistral.


4 DEXTRAL


5 SINISTRAL

2 The shell may show considerable variation even within a single species at a given location or in a sequence in a river basin (Plate 1).

3 The major shell features are illustrated in Figure 9.

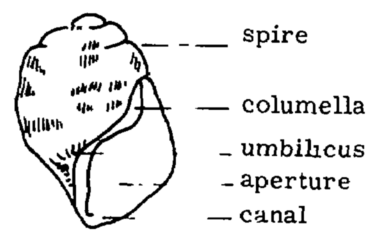


Figure 9

<u>L. g. geniculata</u> <u>L. g. fuliginosa</u> <u>L. g. pinguis</u>


6 down stream form


7 mid-river knobbed form

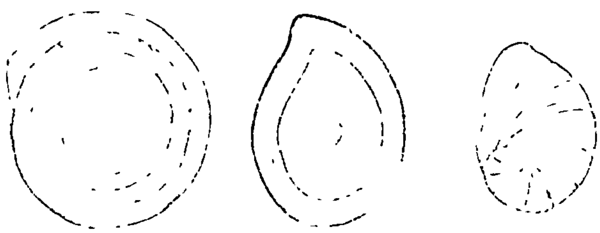

8 smooth headwater form

<u>Lithasia geniculata</u>

Unfortunately, these many varieties were once described as separate species, so many species today have a formidable synonomy. It is no less unfortunate that the majority of today's species have been "lumped," also intuitively.

PLATE 1

B The gilled snails (Streptoneura) have an additional structure, the operculum (10-12), a chitinous plate, which closes the aperture when the body is withdrawn in the shell.



10 multispiral 11 concentric 12 paucispiral

C The body (often called "soft parts" in contrast to the shell) is attached and withdrawn into the shell by the columellar muscle.

1 Foot

The muscular part of the anatomy which protrudes from the shell is called the foot. Typical structures of the foot including the head are shown below·

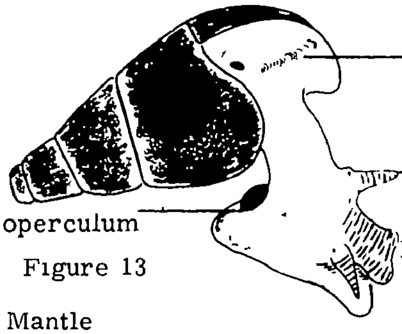


Figure 13

2 Mantle

The thin sheet of specialized tissue which lines the shell and also secretes the shell material during growth is called the mantle. Specialized mantle areas include:

a An external gill or ctenidium composed of a series of plates and highly vascular. Only the order Streptoneura have ctenidia.

b An internal lung cavity with an external opening, the pneumostome which enables them to breathe air at the surface or circulate water and extract the DO. All nonoperculate freshwater snails (of the order Basommatophora-Pulmonata) have this structure.

c Pseudobranch

This respiratory (modified gill) structure is an external extension from the foot and is found in the families Ancylidae and Planorbidae (in the Basommatophora).

3 Remaining parts of the body conform to the internal cavity of the shell and contain portions of the gut and also reproductive systems.

4 Radula

A conspicuous part of the buccal mass (anterior part of head) is the radula, which is one of the most characteristic features of the gastropoda. This toothed structure is used for rasping food from the substrate.

Figure 14

The order Streptoneura have seven teeth per row as shown.

The order Basommatophora, have more than seven (19 to 175).

III ECOLOGY

Distribution of snails is closely related to water chemistry, physical characters of the habitat, geography, and food habits.

A Chemistry

Distribution is related in large measure to dissolved salts, especially calcium carbonate, the essential material for shell construction. Other factors include pH which is associated with and partly determined by the CO_2 content.

B Zoogeography

The gastropod fauna in the United States is very diverse and largely endemic. Such genera as Lymnaea, Helisoma, Gyraulus, and Ferrisia are widely distributed. The Pilidae, for example, are confined to Florida and southern Georgia. Some rivers and river systems have unique faunas.

1 An ecological principle is, "the more extensive a species ecological range, the wider its distribution will be " Examples are these introduced species which in their home ranges have both an extensive range and show continued expansion in their original range.

a Melanoides tuberculata

Afroasian, now found in Texas and Florida (intermediate host for the liver fluke producing Clonorchiasis)

b Thiara granifera

Asian, now also found in Texas and Florida (intermediate host for the lung fluke producing Paragonimiasis.)

c Viviparus japonica

Asian, now widely distributed in United States, especially numerous in Lake Erie.

d Bythinia tentaculata

European, now numerous in northern states and the Great Lakes.

e The first three, Asian species, also exhibit great divergence in shell characters and as a result, these varieties have been described as species for each island and land mass.

Exotics lacking parasitic controls and competition often reach large densities. For example, the first two mentioned (a and b) have reached populations in the San Antonio area of 33 per square inch, and this density was maintained over a period of three years observation. (Murray and Wopschall).

2 Due to changes in water quality, including impoundment, a number of endemic genera and species are either extinct or are endangered because of their limited ecological tolerances

C Water quality requirements of even our common gastropods are still not completely understood. In general, however, gilled snails are seldom found in areas of gross organic pollution, while air breathing snails may be found almost anywhere (unless excluded by other factors). There are so many exceptions to pollution and gastropod relations that generalizations are not meaningful.

D Food

Since most snails are browsers, they are dependent upon periphyton and benthic algae to a large degree

IV COLLECTION

The exploitation of many habitats, secretive habits, and migrations make this group the most difficult benthic invertebrate fauna to collect, next to the bivalves. To put it another way, one must know what species to expect, the particular habitat to search, and the appropriate collecting gear.

V PREPARATION

Although snails usually may be identified without the body and/or the operculum, these are sometimes essential. Therefore, these animals should be relaxed, killed, fixed, and preserved in toto. The first step is the most difficult. A variety of relaxants including Nembutol, menthol, and organic pesticides have all been successfully used Unbuffered formalin will eventually destroy all the shell except the periostracum, thus shelf life of preserved bottom samples is seriously shortened. The remaining periostracum in carelessly preserved collections will be a crumpled mass and unidentifiable.

VI Identification requires precise locality data for the specimen at hand, well preserved material, appropriate keys, and descriptive material for confirming identification

A The gastropods are a relatively simple group to key and have been carefully studied, the great majority being known for years.

1 A real problem here is the confusion resulting from investigators unable to resolve problems in accepted names.

a Pleurocera zonalis Raf. equals Lithasia obovata Say

b Pleurocera canaliculata Say equals Oxytrema canaliculata Say

2 In the Lymnaeidae the problem involves generic and/or subgeneric classification as well as what constitutes a species

3 For these reasons specific identification should involve consultation with a specialist in the particular family involved. Then one should remember that taxonomy is far from settled in these groups.

FRESHWATER MOLLUSCA

Part 3. Bivalves

I The great majority of living bivalves are lamellibranchs, that is, they have enormously enlarged gills. These ctenidia, like those in a few genera of mesogastropods such as Pleurocera, are enlarged by elongation of the filaments so that, together, adjacent filaments form a lamella.

A These lamellae are more than sufficient for respiration. They are in fact the major organ of food collection in these filter-feeders.

B A water current through the mantle cavity is created by the lateral cilia. This flow passes between the filaments of the ctenidium from the inhalant siphons to the exhalant siphon. The particulate matter remaining on the inhalant face of the gill, the frontal cilia and mucus are used to make chains or boluses of this material that then pass on to the mouth.

C The amount of water passed through this system is relatively large, for example, a small 20 gm clam can pump nearly four liters per day. Thus, clams concentrate heavy metals, radionuclides, pesticides, bacteria, and viruses, etc.

D Wastes from this system include feces and pseudofeces.

1 Fecal deposits are discharged from the anus into the exhalant siphon. These tightly bound and mucous connected strings may smother older generations in a deposit. They also may contain significant amounts of nutrients and/or pesticides.

2 Pseudofeces are mucous connected sheets which are rejected solid materials collected on the gills in the feeding process. By vigorous mantle contractions of the shell these are discharged through a mantle opening.

Pseudofeces may form significant benthic deposits (phosphorus in biogeochemical cycles) which alter irrigation canal hydraulics. Pediveligers may also be trapped in this mass.

II The structure of all five families, all belonging in the same subclass of freshwater bivalves is basically the same.

A Size and reproductive habits differ in each family.

Table 1

PHYLUM MOLLUSCA - CLASS BIVALVIA
SUBCLASS EULAMELLIBRANCHIA
ORDER SCHIZODONTA
SUPERFAMILY UNIONACEA
1 FAMILY MARGARITIFERIDAE
2 FAMILY UNIONIDAE
ORDER HETERODONTA
SUPERFAMILY SPHARIACEA
3 FAMILY SPHAERIIDAE
4 FAMILY CORBICULIDAE
5 FAMILY MACTRIDAE

1 The mussels (Margaritiferidae and Unionidae) are classed in the superfamily Unionaceae.

a They exhibit schizodont tooth structure.

b They reach a size of up to 9 inches.

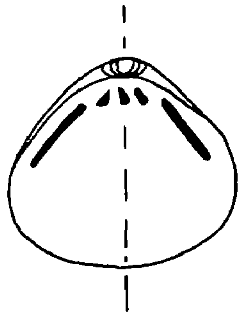
c Longevity is relatively long, up to > 90 years.

d Many species are of commercial value for shell export to Japan. The shells being processed into nuclei for production of cultured pearls in the Japanese pearl oyster.

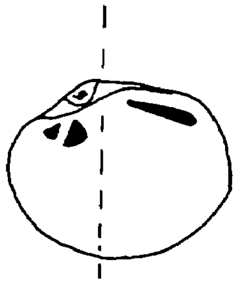
e Commercially valuable species have accepted and widely used vernacular names, such as pigtoe, maple-leaf, monkey-face, pocketbook, etc.

f Unionids are obligate fish parasites. The glochidia (homologous with veliger larvae) are discharged from the gills into the water, clamp onto the gills or fins of the host fish. After a short parasitic development period, they drop off to begin benthic life.

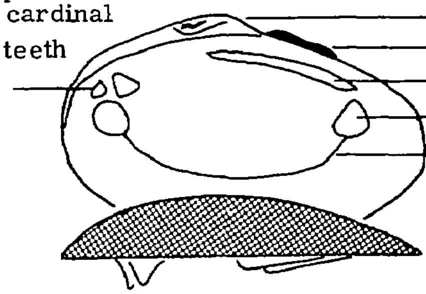
Corbicula is intermediate in size and longevity.



15 heterodont



16 schizodont



17 Interior and Top View of Mussel Shell

2 Fingernail clams (Sphaeriidae) and an introduced species Corbicula (Corbiculidae) have heterodont teeth (cardinals and laterals) and belong to the same superfamily, Sphaeriacea.

a Up to 65 mm in shell length

b Longevity is approximately five to ten years.

c They are canal clogging, pipe clogging, etc., organisms of serious magnitude.

d The veliger larvae are discharged to the water in large numbers. Pelagic larvae are then freely carried into raw water systems. A byssus and highly specialized foot allows them to establish a sessile habit in flowing conduits or form loosely aggregated masses in the substrate.

3 The remaining family, Sphaeriidae, are the smaller and shortest lived group.

a Size is from two to twenty mm, but usually not more than ten mm.

b Longevity is up to two years.

c The genera Sphaerium, Musculium, and Eupera are nearly equipartite, and thus are called "fingernail clams." The genus Pisidium is inequipartite and these are called "pea shells."

d One to twenty individuals develop within the marsupial gills. These are released as juveniles.

III Shell and body are structurally and developmentally a single entity although they are often described as though the two valves and the ligament are of different origin.

A Basic shell structures are as shown.

1 Attachment of muscles and other soft parts are clearly marked on the shell as impressions. The largest bivalve muscles are the adductors which pass through the mantle tissue and are attached to each valve. The elasticity of the ligament and several kinds of hydraulic systems serve as antagonists for the adductors.

2 Shell teeth are simply shell projections which serve as a fulcrum for anticulation of the shell valves.

3 The shell is composed of three layers

a The outer or periostracum is mainly organic.

b The mid or prismatic layer is composed of minute prism-like blocks of calcium carbonate.

c The inner or nacre (mother of pearl) is composed of alternating layers of calcium carbonate and an organic substance. The species with the thickest nacre and silver-white sheen were originally the most valuable for the pearl button industry. This is also true for the cultured pearl industry which now utilizes our entire commercial harvest for processing into "nuclei" to be inserted into the pearl "oyster" for cultured pearl production.

B The body mass is enveloped by the mantle. Siphons, gills, and foot are major sections of the body.

1 The mantle functions in respiration and shell formation.

2 The ventral margins of the mantle are fused at various places.

a Openings for the foot and byssus.

b Fused to form various siphonal structures, such as exhalant and inhalant siphons.

C The foot is a highly elastic organ which can be extended knife-like into the substrate, expanded at the tip to serve as an anchor, then the hatchet shaped shell is forced into the substrate as the foot contracts.

1 Some species are more active burrowers than others.

2 Although many must have the siphons reaching the substrate-water interface, others siphon while burrowed in gravelly and loose substrates (benthic and hyporheal).

D Gills are simply modified folds of the mantle and are an elaborate organ for respiration, feeding, and often maternal brooding of eggs and larval stages.

E These animals are considered acephalic, that is, headless which confused early scientists trying to describe anterior or posterior shell features. The head is at least represented by the mouth. The siphons are located at the posterior end, and thus, the animal's anterior end is directed downward or into the substrate. Bivalves are the only molluscs without a radula.

IV The ecology of most of our freshwater bivalves are poorly known, although nearly all of our species were described by early naturalists.

A As in gastropods, the calcium carbonate shell is a reflection of water chemistry. For example, the shell can accumulate large quantities of heavy metals. Where the periostracum has been worn off or damaged, the underlying calcium carbonate may be severely eroded.

B Dispersal of bivalves has been noticeable.

1 There are a number of the smaller species of Sphaeriidae which are cosmopolitan or which have been introduced from both sides of the Atlantic.

2 An exotic which has been particularly significant as a nuisance organism is the "Asian clam," Corbicula manilensis.

3 At least one species of Unionacea is circumboreal, Margaritifera margaritifera

4 The United States has a pronounced regional Unionid fauna as shown by the map. The southern Applachians have a unique fauna. Many species are already extinct and others are in danger due to man-induced-changes in the ecosystem.

C Changes in water quality are difficult to evaluate in the group since the Unionids require a fish host, often specific. It follows that changes in fish fauna will also be reflected in mussel populations.

1 Mussels have generally been regarded as indicators of clean water, however, it has been shown that several species, including Megalonais gigantea and Amblema plicata have remnant populations in streams subjected to a variety of effluents. Therefore, generalizations would not be meaningful.

2 It should also be obvious that adult molluscs (remember also that they may reach upwards of 50 years) which can remain closed and respire anaerobically can withstand severe conditions during limited periods. Reproduction and maintenance of such populations should be evaluated.

3 The absence or presence of appropriate fish hosts must be evaluated. These factors (1 and 2 above) make mere presence of adult individuals of mussels meaningless in water quality investigations.

D Food habits need careful study to determine what elements of the food string is utilized. Although some sorting of food does take place on the palps, a variety of phyto and zooplankton, bacteria, and various suspended and dissolved organic and inorganic materials pass through the gut, with varying degrees of utilization.

V Qualitative and quantitative collection of Unionids or mussels is achieved with difficulty. The rarer species are often collected by examining the shell piles accumulated by foraging muskrats.

A In large streams and lakes scuba diving techniques using an iron frame for selection of quadrats yield quantitative results.

B Small streams are difficult to sample.

1 Migratory movements of some mussels make them relatively inaccessible.

2 Special techniques are needed for collecting juveniles.

VI Since anatomical and reproductive features are useful characters for identification, bivalves should be relaxed, killed, fixed, and properly preserved.

A For relaxation, menthol crystals sprinkled on the water surface of the water containing them may work.

B Sphaeriids are easily relaxed by placing them in an acceptable water in a petri dish and adding a small granule of pentachlorophenol.

C If naiads are left out on a counter, they can be "pegged" when the shell gapes. The peg allows the preservative to reach the soft parts, and also makes it easier to insert a scapel to sever the adductor muscles.

D An unbuffered formalin solution will effectively decalcify the valves, destroying a specimen.

VII Final identification to species should be left to the specialist. Although there are virtually no undescribed species, the systematics of this group are unstable. The main problem involves the matter of interpretation of taxonomic rules, including priority.

VIII The larger group of bivalves classed as Unionidae have a variety of names for the group-including mussels, freshwater clams, pearl button clams, unionids, pearly naiads, nayades, najades, etc. The most accepted group name today is the term Naiads.

Nearly all Naiads have common names which are specific. These are the same for north or south, since they evolved during the pearl button era. They were marketed under such names as pistol grip, washboard, three ridge, monkey face, etc.

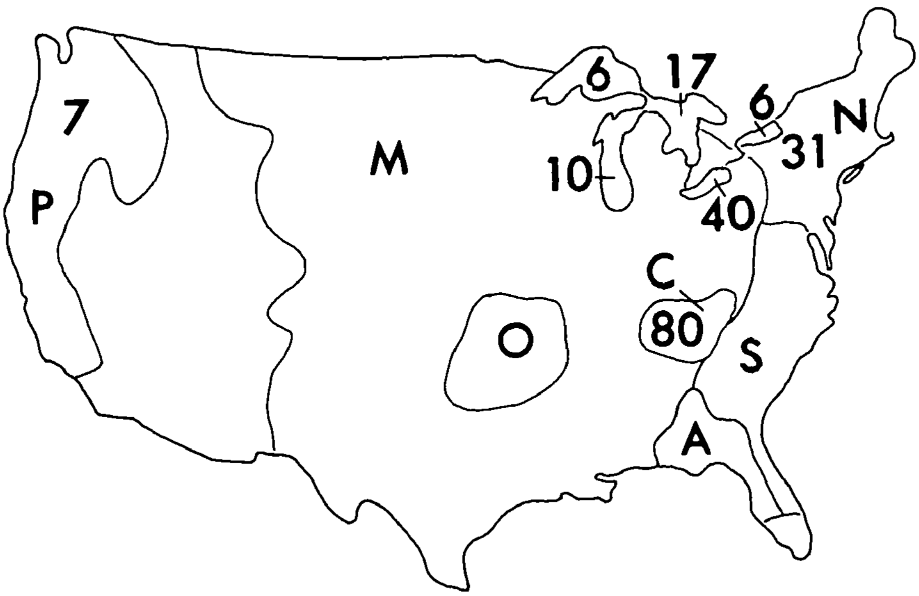


Figure 18. MUSSEL REGIONS OF THE UNITED STATES (after van der Schalie)

numbers = app. no. of species P - Pacific M - Mississippian O - Ozarkian C - Cumberlandian N - North Atlantic S - South Atlantic A - Appalachicolan

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FISHES

I INTRODUCTION. What is a fish?

A A fish is a gill-breathing aquatic vertebrate with fins (exceptions noted).

B Other Aquatic Vertebrates

1 Amphibia - frogs, toads, salamanders

a Modern amphibia do not have scales.

b Tadpole stages easily recognized.

c Pollutional significance not studied to date. Frogs often observed in polluted waters but not aquatic salamanders

2 Reptilia - snakes and turtles

a Relatively independent of water quality as long as it is not irritating.

b Carnivorous types would be starved out of polluted areas for lack of food.

3 Mammalia - muskrats, beavers

a Generally inhabit wilderness areas where heavy pollution is not a problem.

II STRUCTURE AND PHYSIOLOGY

A Fins

1 A typical fish has two sets of paired fins, the pectoral and pelvic, comparable (homologous) to our arms and legs respectively. Certain ancient fish could walk on their lobe-like fins, and some specialized modern forms likewise

2 Unpaired dorsal, anal, and caudal or tail fins, complete the fin structures

3 Any or all of these may be missing, and fleshy extra fins such as the "adipose" fins of trout and salmon, catfishes, etc., may appear. Extra paired fins too are known. The dorsal fin is often divided into two or more sections known as 1st, 2nd, 3rd, etc., dorsal fins.

4 Fins may be supported by soft rays or stiff spines or both.

B The body of a typical fish is covered with scales.

1 Four types of scales are recognized

a The most primitive are bony plates bearing tooth-like projections as found in sharks and rays

b Smooth bony plates such as those of the gar and dogfish are somewhat higher in specialization.

c Thin smooth roundish "cycloid" scales are characteristic of the more primitive of the modern "bony" fishes like herring or trout.

d Roundish scales with tiny spines or cteni are characteristic of the highest fishes like the black basses. These are called ctenoid scales

2 Cycloid and ctenoid scales are nonliving material like hair or fingernails, covered with a thin layer of living tissue cells. This tissue is easily injured as by handling a fish with dry hands

C Respiration of a typical fish is by means of blood gills (Cf tracheal gills of insects)

1 Gills, like lungs, are a device for bringing the blood into close proximity to the environment.

2 Certain ancient fishes and their modern descendents, the lungfishes, breathed air Some of the modern bonyfishes like certain catfishes also have a limited air breathing capacity.

D The maintenance of a constant internal osmotic pressure of their body fluids in competition with their environment is a problem which fishes have that terrestrial animals with their waterproof skins do not have.

1 The slime covering body and gills is an important part of the regulatory mechanism

2 Marine fishes live in an environment that tends to dehydrate the body Consequently water appears to be swallowed from time to time in order to replace that lost through the gills and general body surface. This may make marine fishes more susceptible to some toxic substances which would then be taken internally instead of simply contacting the skin externally.

3 Freshwater fishes live in an environment with an osmotic pressure far lower than that of body fluids. Toxic substances would then be less likely to be taken internally except with food.

III REPRODUCTION AND DEVELOPMENT

A Although almost infinite variety exists, most fishes lay their eggs externally in the water, at which time the male showers them with milt Such species are said to be oviparous. In some species such as the familiar guppy however, fertilization is internal and the eggs develop and hatch in the mother's body. No nourishment is known to be transmitted from mother to developing young. Such species are said to be ovoviviparious. The young are thus "born alive."

1 Most fish eggs hatch before all the food material stored in the egg as yolk is used up. Embryonic development is still going on and they are truly in a larval or pre-adult condition. During their first hours or days of free life, they are thus still independent of their environment for food This early stage is known as yolk sac fry or simply sac fry.

2 The young continue to be called fry, or advanced fry until they approach an inch in length, when they are referred to as fingerlings.

3 Fry which differ greatly from the form of the adult may be referred to as larvae.

B Some fish lay their eggs in the same general location in which they live as adults Others travel to some distant place, such as from lakes or rivers up into small streams, from deep water to shoal, from the ocean to fresh water, from fresh water to the ocean, etc These are called breeding migrations.

1 Fish that normally live in freshwater and travel to the ocean to reproduce are called catadromous. The freshwater eel is the best known example.

2 Those that live in the sea and lay their eggs in freshwater are called anadromous Striped bass, shad and certain other herrings, and the salmons are well known examples. Occasionally, a group of anadromous fish will get lost in the inland waters and not be able to find its way back to the sea. These are called landlocked varieties and are usually somewhat smaller than their non-lan-locked relatives

3 Pollution or other factors which either block a breeding migration or destroy a spawning bed may completely destroy

a species, even though the adults in their natural habitat are untouched.

IV CLASSIFICATION OF FISHES

Fishes may be classified or grouped in many different ways.

A Food, Feeding Habits and Ecological Interrelationships

Fish, like many other animal groups, include carnivores, herbivores, and detritus feeders or scavengers

1 Scavengers may specialize on bottom feeding like certain suckers, carps, and catfishes. Others may take any organic matter they can find, wherever they can find it. Scavengers are often provided with barbels or feeders which help in locating food, especially in turbid water.

2 Herbivores may feed on the larger, vascular plants as some carps or they may specialize on the microscopic phytoplankton, in which case they are called plankton feeders. Plankton feeders usually have weak mouths and fine gill rakers for straining the plank ton out of the water.

3 The carnivorous or predatory species feed essentially on living animals They may specialize on invertebrates or other fish, in which case they may be called piscivorous.

a Piscivorous fish usually depend essentially on eyesight for locating their food, and hence turbid water is a handicap.

b The carnivorous fish in general include most of the game fish.

c Small species of fish which are not used directly by man but are used extensively as food by piscivorous species are often referred to as forage fish.

B Classification with reference to their desirability or to mode of use by man has been widely used An example of such a system is as follows

1 Commercial - those that occur in sufficient quantities to support a fishery.

a Food fishes: white-fish, salmon, cod

b Product fishes sharks, blue back herrings

2 Game or sport - those captured essentially for sport. Many species fall into both this and the commercial categories such as the trouts, black-basses, striped bass, etc.

a Gamefish are sometimes considered to be those which are of interest to man only for the catching, as the tarpon.

b Fish which are taken, even though in sport, but which are also eaten are then called panfish. Sometimes panfish refers only to the smaller of the edible gamefish.

3 Rough fishes are those such as the gars, and the bowfins, which are of little or no use to man. Some, such as the carp, are classed in different groups in different regions according to local custom.

C A classification developed with reference to standard methods of reporting fish population data for reservoirs is as follows (Surber '59)

Group 1 Predatory Game Fish - bass, crappies, trout, etc

Group 2. Non-predatory Game Fish - sunfish, rock bass, perch, etc.

Group 3. Non-predatory Food Fish - carp, drum, buffalo, suckers, bullheads, etc.

Group 4 Predatory Food Fish - catfish, gar, bowfin, etc.

Group 5 Forage Fish (Non-predatory) gizzard shad, threadfin shad, Gambusia, minnows, etc.

D The scientific classification system for fishes lists many thousands of species Three great groups of living fishes occur in this country. the Agnatha or jawless fishes, the Chondrichthyes or cartilaginous fishes and the Osteichthyes or (modern) boney fishes. Some additional groups occur in other parts of the world.

1 The jawless fishes are represented in fresh water by the lampreys, which have in recent years invaded the Great Lakes from the sea and wrecked havoc on the native species.

2 The cartilaginous fishes are the sharks, skates, and rays, primarily a marine group

3 The vast majority of fishes with which most of us are familiar belong to the Osteichthyes or bony fishes, a few typical families are listed below·

a The family Acipenseridae or sturgeons are a primitive group, famed for their roe which is sold as caviar. More or less covered with large, bony plates. Formerly extremely abundant and large in size

b Family Lepisosteidae - the gars. These Voracious fish are covered with hard, enamel-like, rhomboid scales. Some species may grow to great size. Widely regarded as "trash" fish

c The family Salmonidae includes the trouts, salmons, whitings, and graylings. Scales are cycloid and always small, an extra or adipose fin on the back, eggs are very large, favored by cold water. In the Pacific Salmon, but one set of reproductive cells is formed in the life of the individual, which therefore dies after spawning once.

d The family Catostomidae is the suckers. The head is naked of scales, jaws toothless, mouth usually protractile, lips generally thick and fleshy Feed on plants and small animals.

e The family Cyprinidae is the carp-dace-minnow group. Here too the head is naked, and the body usually scaled. Ventral fins usually well back. Teeth are lacking in the jaws. Certain bones in the back of the throat known as the pharyngeals are strongly developed however, and bear from 1 to 3 series of teeth which are often of importance in identification. Upwards of 1800 species, abundant where present at all, both in numbers and variety Generally small in size although Leucosomus corporalis the chub, roach, or fallfish may reach a length of 18 inches in the east, and related species 5 to 6 feet on the west coast. Because of the many similar species, this is one of the most difficult groups in zoology to identify to species

Two genera, Cyprinus which includes the common carp, and Carassius including the goldfish have been introduced and become widely established. Both are native to China Other introduced Cyprinids have so far not become widely established.

f Family Ictaluridae, the freshwater catfishes. Body more or less elongate, naked Eight barbels or feelers in head region. Dorsal fin short, an adipose fin behind. First ray of dorsal and pectorals developed as stout spines. Many excellent food fish. Very tenacious of life.

g Family Centrarchidae - sunfishes and freshwater basses. Scales ctenoid Dorsal fin continuous but may be in two sections, the anterior spined, the posterior rayed Generally carnivorous. Typical of eastern North America but have been widely introduced in other areas Nest builders.

4 Additional well known families of bony fishes are listed below.

Polyodontidae	-	paddlefishes
Amiidae	-	bowfins
Gasterosteidae	-	stickelbacks
Cyprinodontidae	-	killifishes
Serranidae	-	sea basses
Ictaluridae	-	freshwater catfishes
Percidae	-	perches and darters
Cottidae	-	sculpins
Atherinidae	-	silversides
Clupeidae	-	herrings
Osmeridae	-	smelts
Salmonidae	-	whitefishes, trouts, etc.
Anguiledae	-	eel
Poeciliidae	-	guppies, mosquito-fishes
Gadidae	-	cods, hakes, burbots
Esocidae	-	pikes and pickerels
Sciaenidae	-	drums

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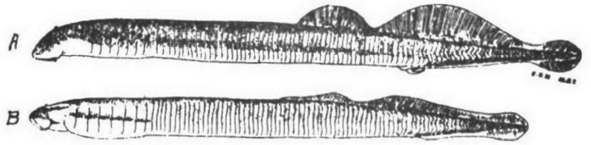
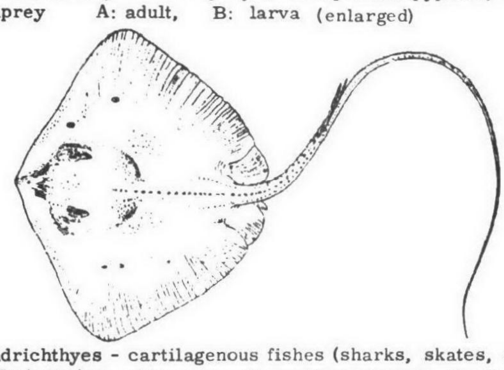
This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

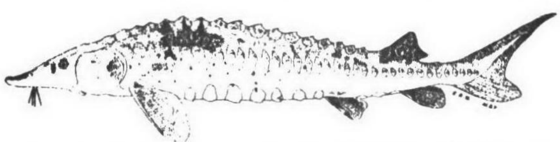
SOME PRIMITIVE FISHES



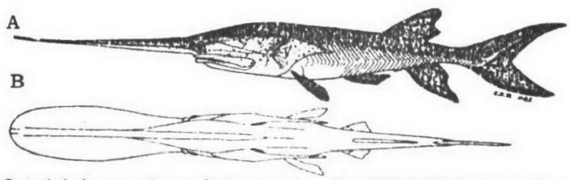
Class Agnatha, jawless fishes (lampreys and hagfishes) - Family PETROMYZONTIDAE, the lampreys. Lampetra aepyptera, the Brook Lamprey A: adult, B: larva (enlarged)



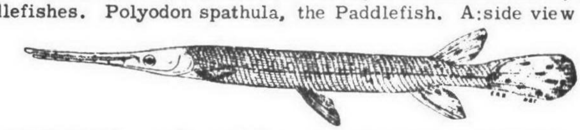
Class Chondrichthyes - cartilagenous fishes (sharks, skates, rays) Family DASYATIDAE - stingrays. Dasyatis centroura, the Roughtail Stingray



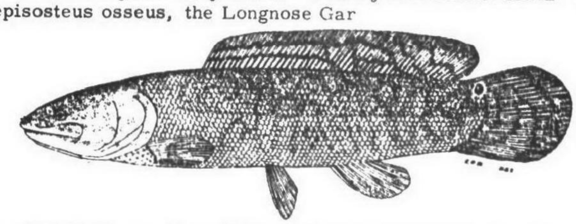
Class Osteichthyes - bony fishes - Family ACIPENSERIDAE, sturgeon. Acipenser fulvescens, the Lake Sturgeon



Class Osteichthyes - bony fishes - Family POLYODONTIDAE, the paddlefishes. Polyodon spathula, the Paddlefish. A:side view B:top view



Class Osteichthyes - bony fishes - Family LEPISOSTEIDAE - gars Lepisosteus osseus, the Longnose Gar



Class Osteichthyes - bony fishes - Family AMIIDAE, bowfins Aniia calva, the Bowfin

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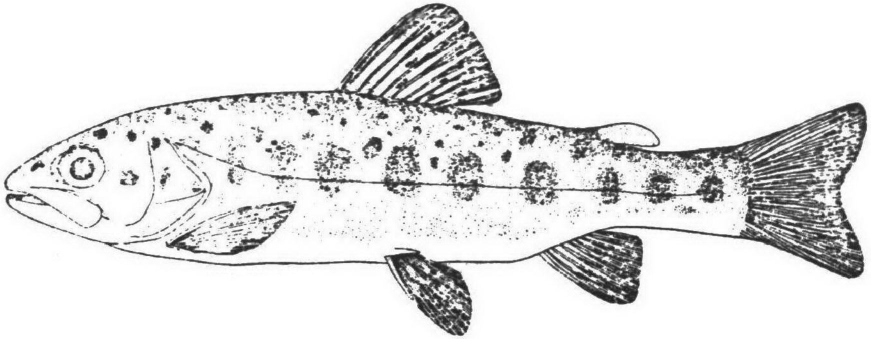
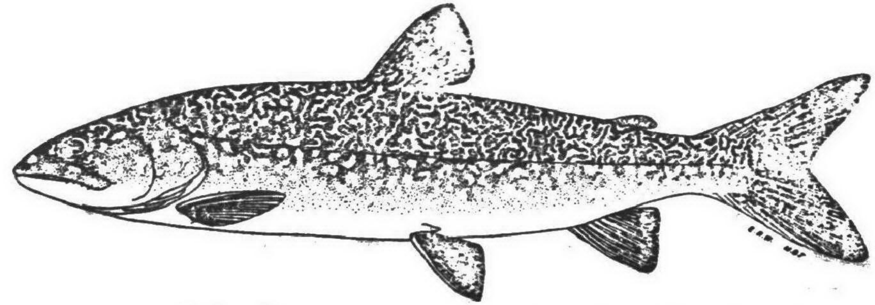
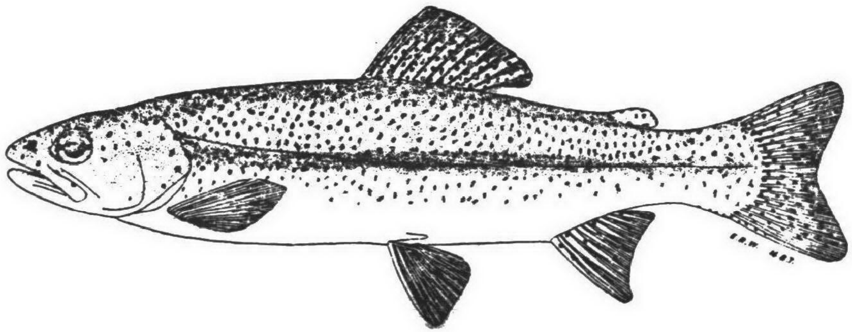
Family SALMONIDAE



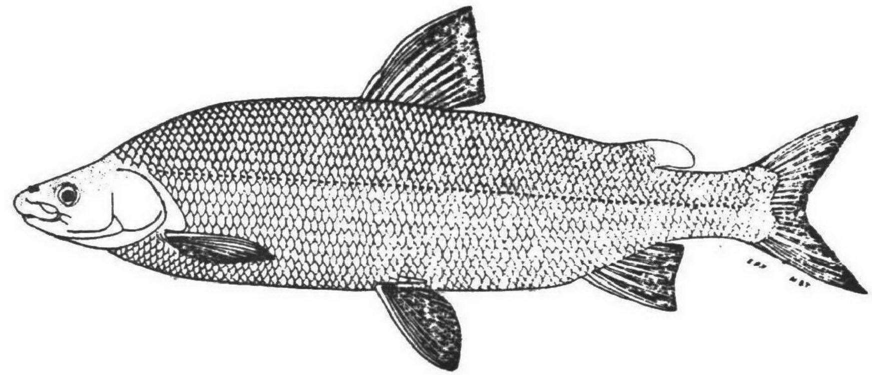
Salmo trutta - brown trout



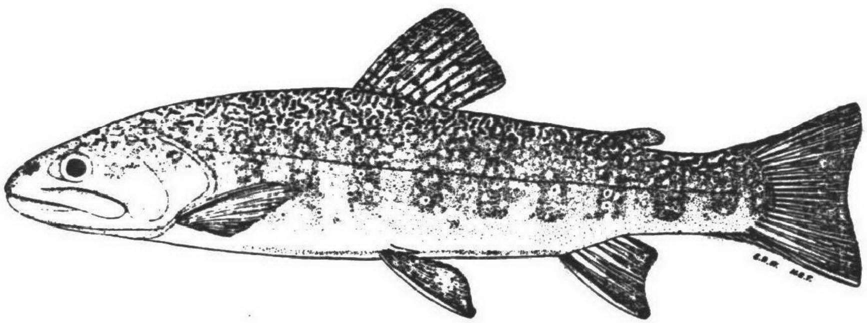
Salvelinus namaychush - the lake trout



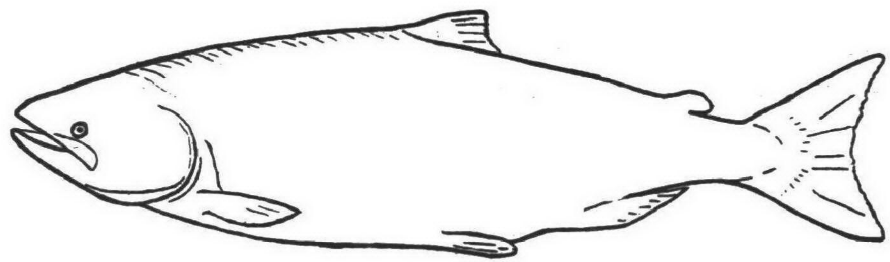
Salmo gairdneri - rainbow (or steelhead) trout



Coregonus clupeaformis - lake whitefish



Salvelinus fontinalis - brook trout



Oncorhynchus tshawytscha - the chinook salmon

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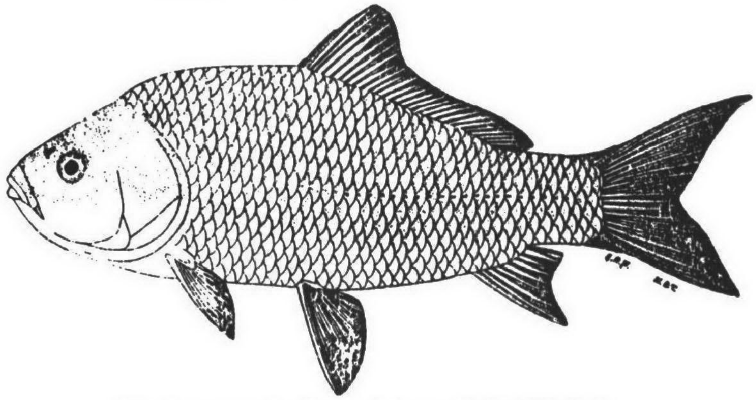
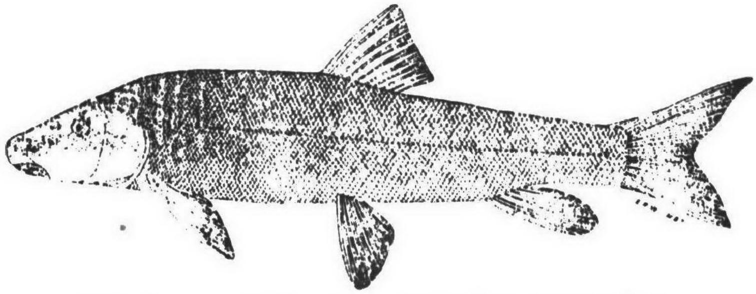
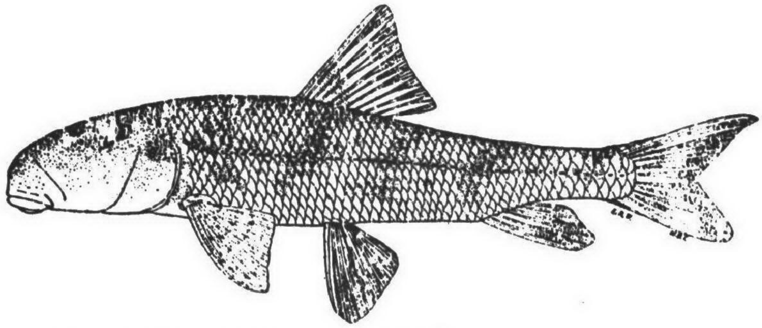
Family CATOSTOMIDAE - the suckers



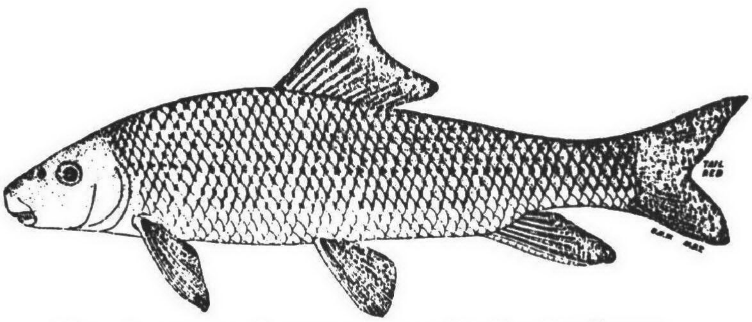
Ictiobus cyprinellus - bigmouth buffalofish



Catostomus catostomus - eastern longnose sucker



Hypentelium nigricans - hog sucker



Moxostoma aureolum - northern shorthead redhorse

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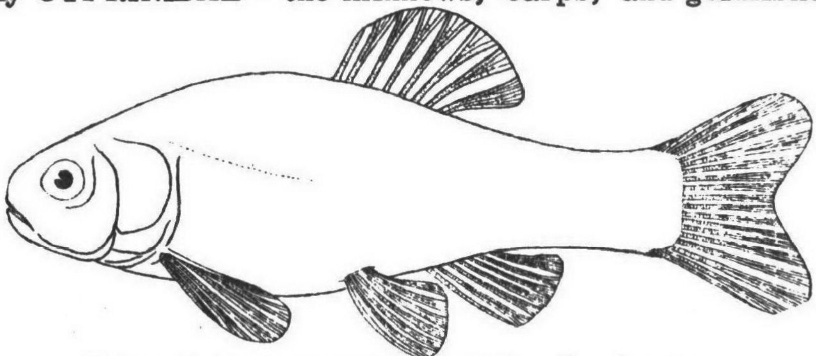
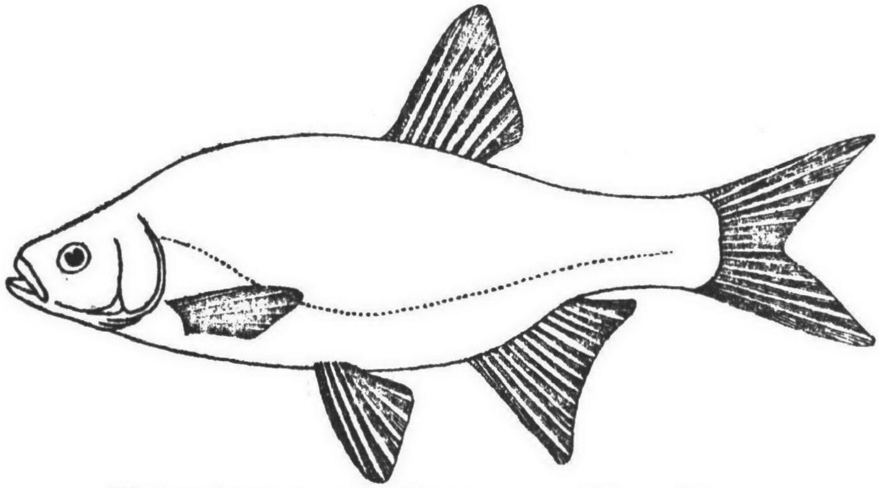
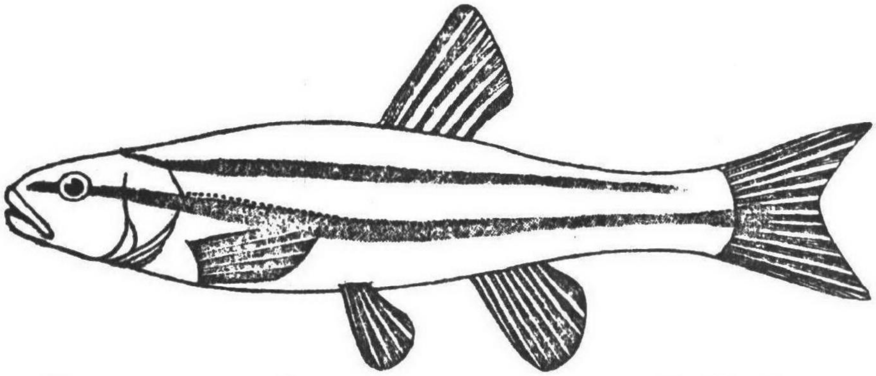
Family CYPRINIDAE - the minnows, carps, and goldfishes



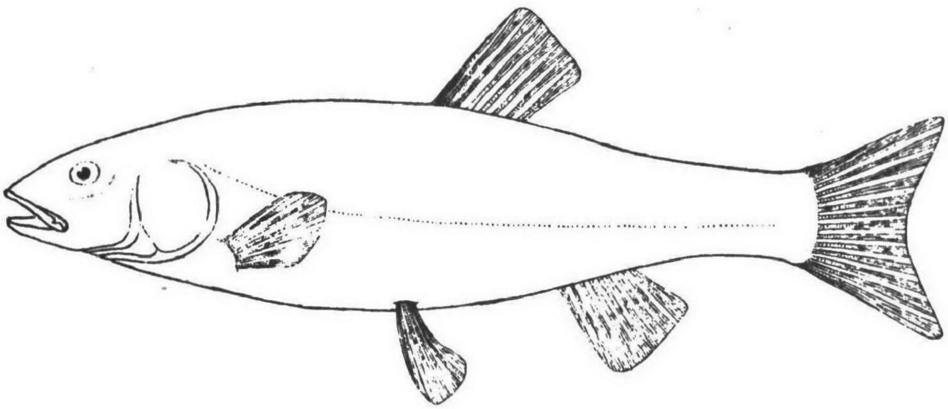
Pimephales promelas - fathead minnow



Notemigonus crysoleucas - golden shiner

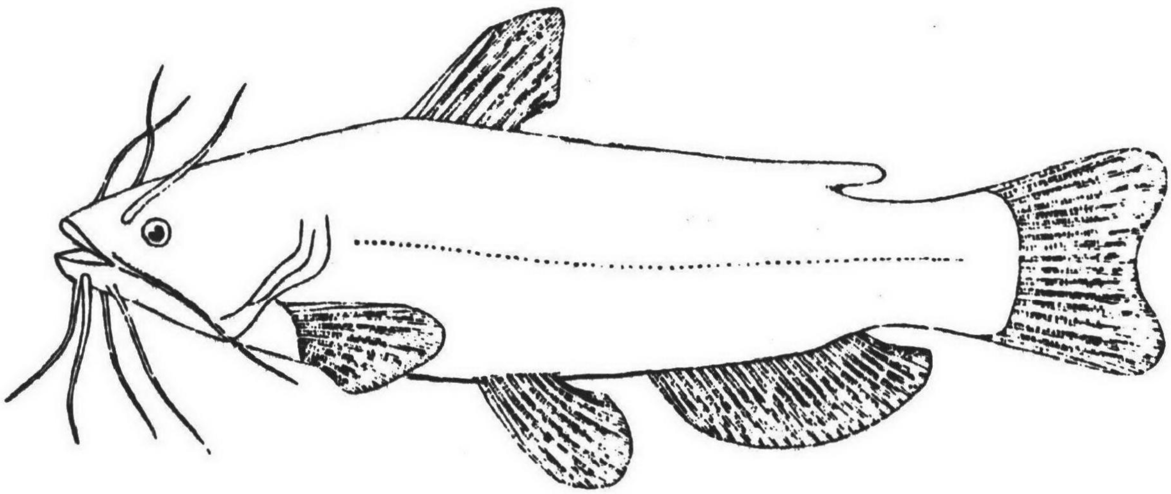


Chrosomus erythrogaster - southern redbelly dace

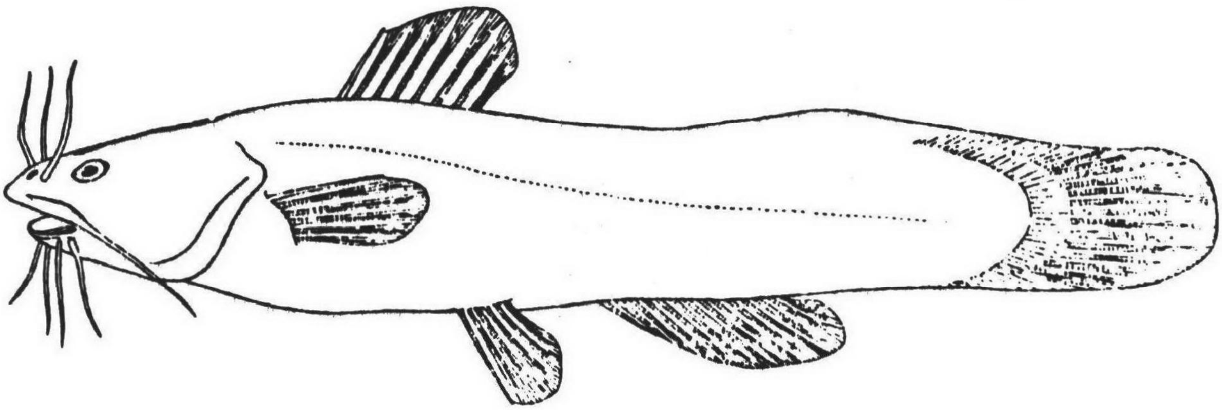


Semotilus atromaculatus - creek chub

Family ICTALURIDAE - the freshwater catfishes



Ictalurus nebulosus - brown bullhead



Noturus insignis - margined madtom

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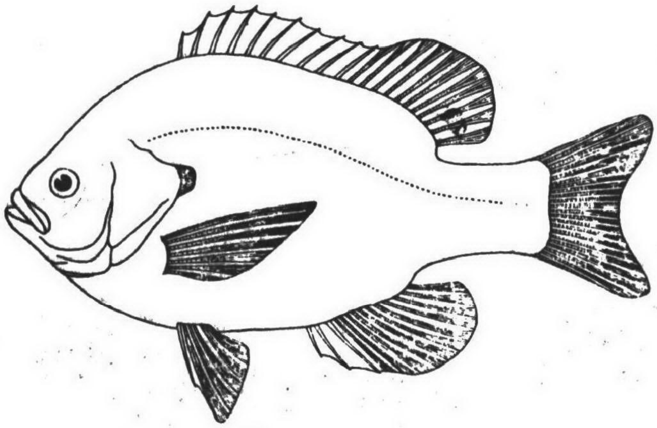
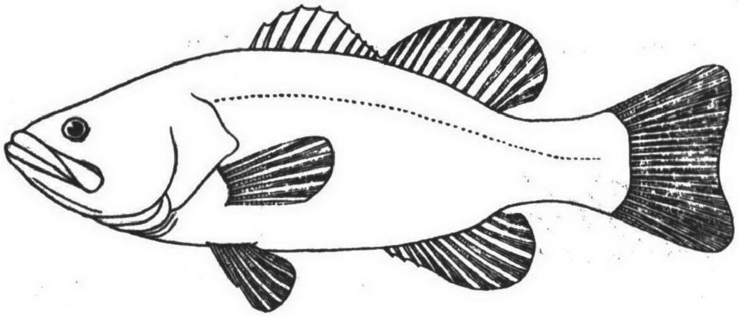
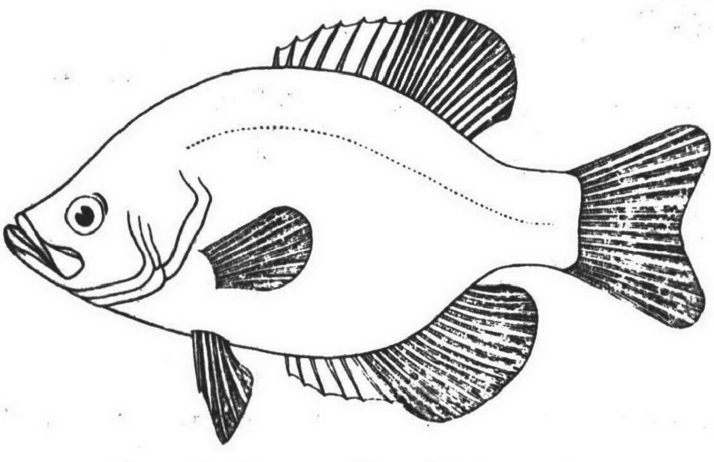
Family CENTRARCHIDAE - the sunfishes



Lepomis macrochirus - bluegill



Micropterus salmoides - largemouth bass



Pomoxis nigromaculatus - black crappie

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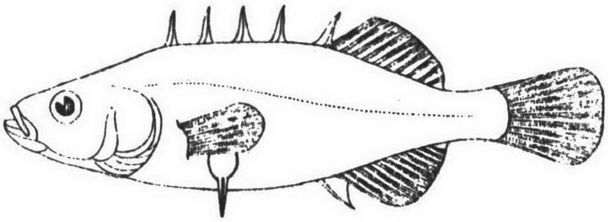
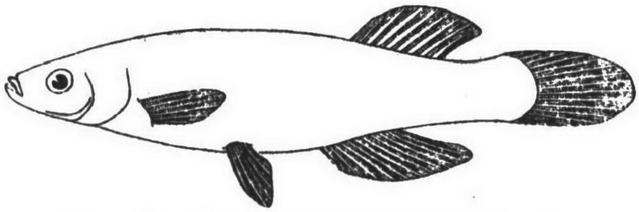
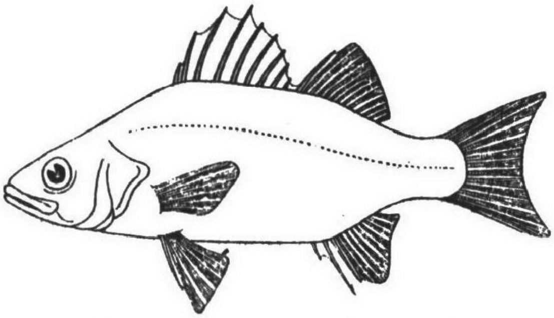
TYPES OF BONY FISHES I



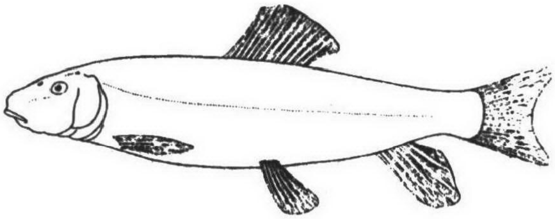
Family GASTEROSTEIDAE, the Sticklebacks. Eucalia inconstans, the brook stickleback



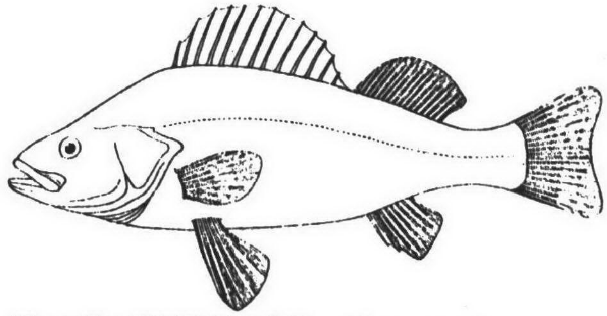
Family CYPRINODONTIDAE, the Killifishes. Fundulus notatus, the blackstripe topminnow



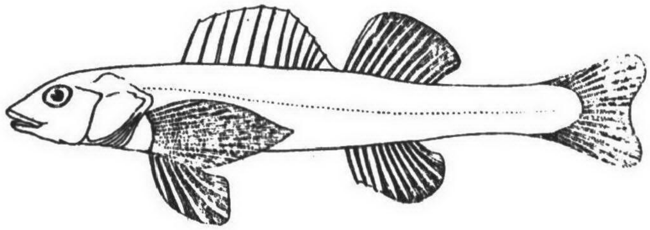
Family SERRANIDAE, the sea basses. Roccus americanus, the white perch



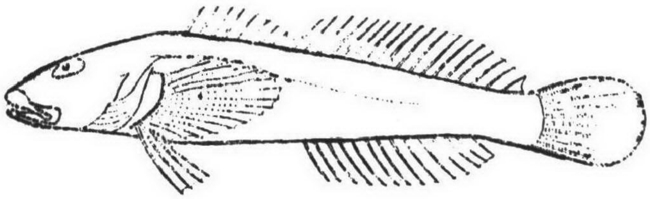
Family CATOSTOMIDAE, the suckers. Catostomus commersonii, the white sucker



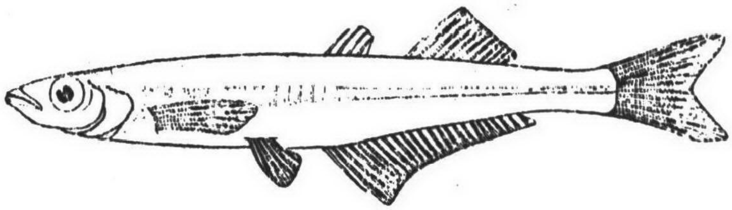
Family PERCIDAE - the perches. Perca flavescens, the yellow perch



Family PERCIDAE, the perches. Etheostoma nigrum, the johnny darter



Family COTTIDAE, the sculpins. Cottus bairdii, the mottled sculpin



Family ATHERINIDAE, the silversides. Labidesthes sicculus, the brook silverside.

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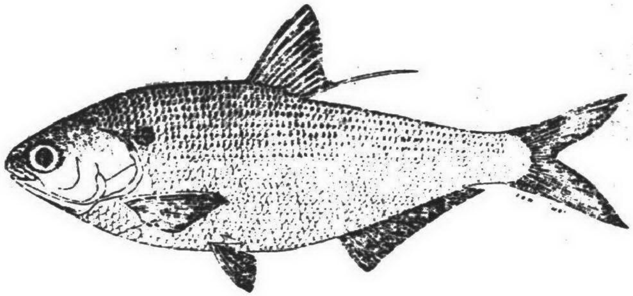
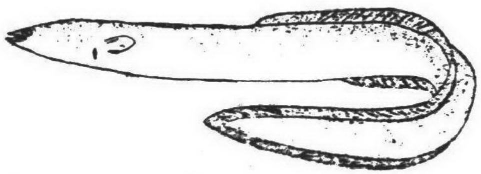
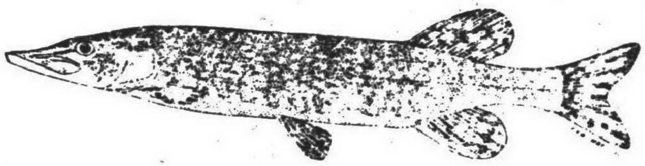
TYPES OF BONY FISHES



Family CLUPEIDAE - herrings
Dorosoma cepedianum - the eastern gizzard shad



Family ANGUILLEDAE - freshwater eels
Anguilla rostrata - the American eel



Family ESOCIDAE - pikes
Esox lucius - the northern pike

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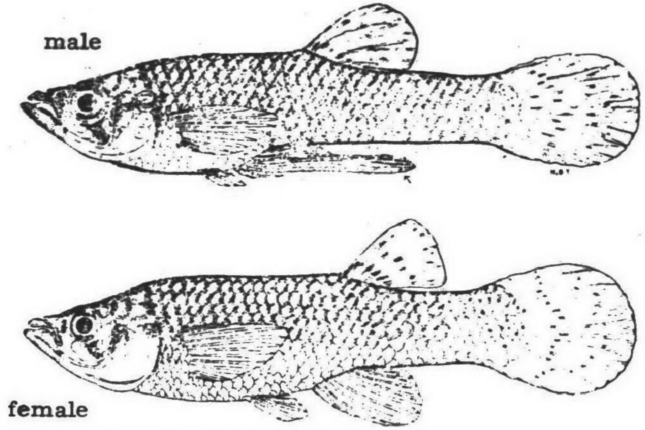
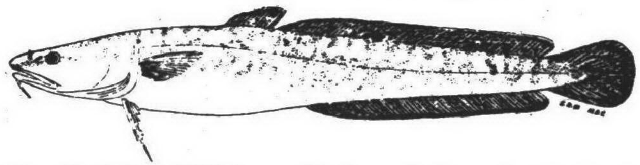
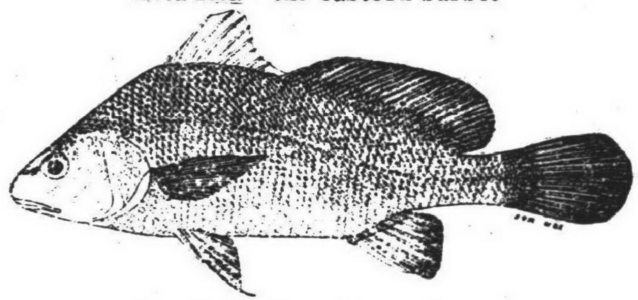
BI. AQ. pl. 9m. 6.60



Family POECILIIDAE - livebearers
Gambusia affinis - the mosquitofish

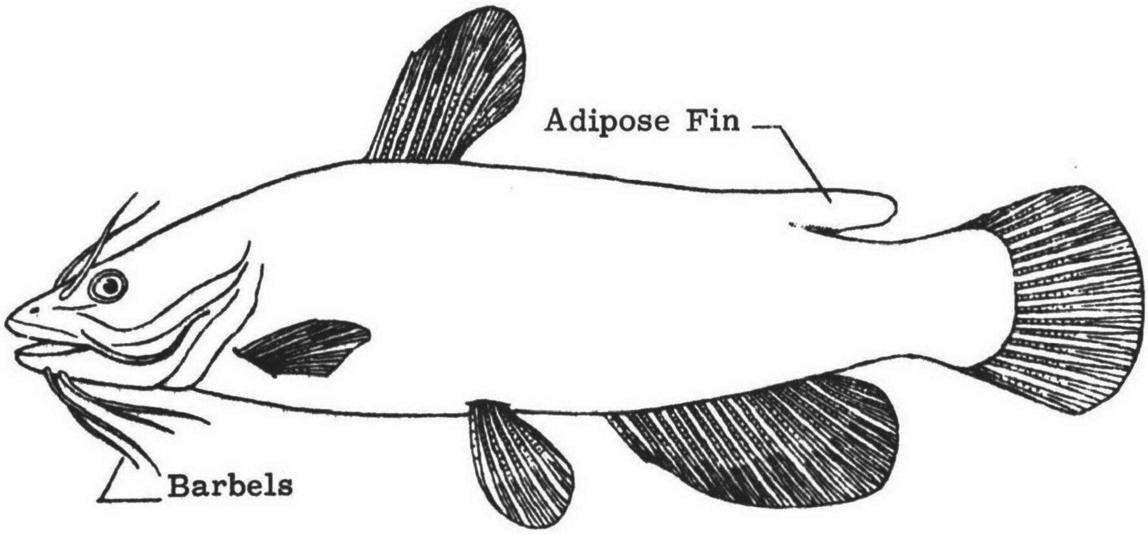


Family GADDIDAE - codfishes, hakes, haddock, burbot
Lota lota - the eastern burbot

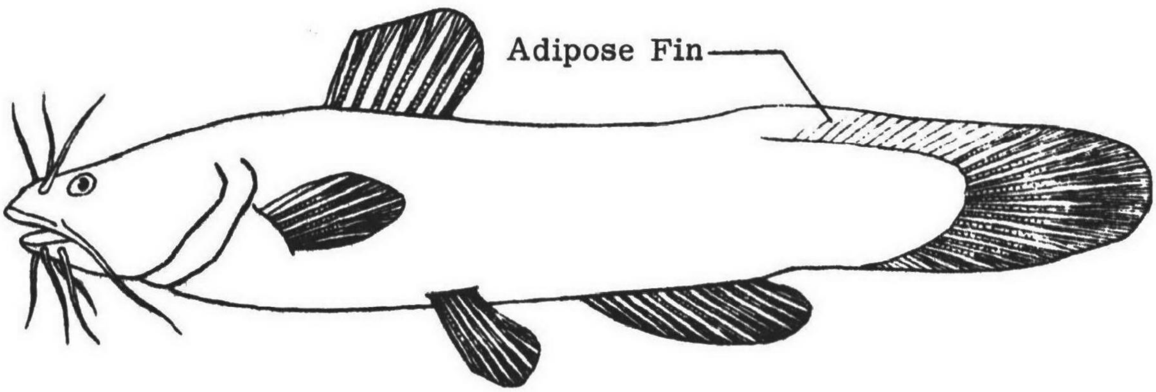


Family SCIAENIDAE - drums
Aplodinotus grunniens - the freshwater drum

ADIPOSE FINS - in catfishes



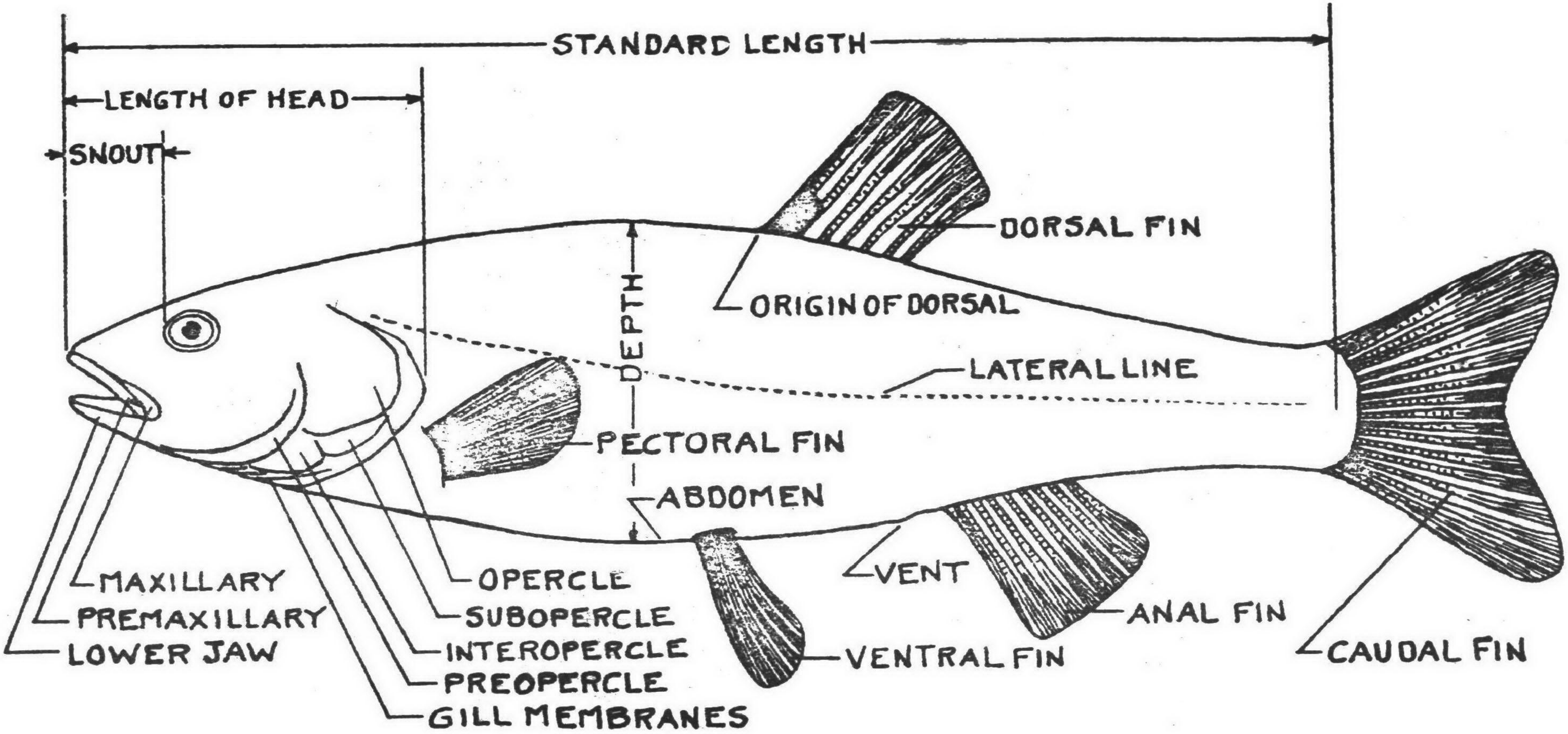
The Adipose Fin does not extend to the Caudal Fin
(Ictalurus nebulosus)



The Adipose Fin extends to the Caudal Fin
(Noturus insignis)

Reproduced with permission; Hart, Doudoroff and Greenbank, 1945.

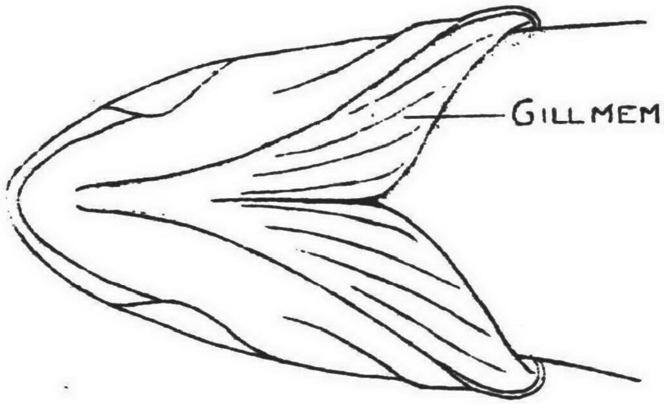
BI. AQ. pl. 9e. 6. 60



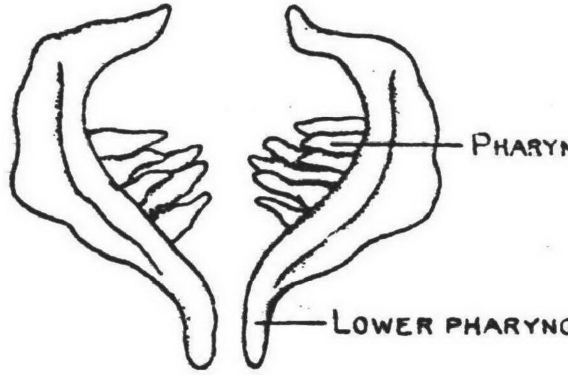
A SOFT-RAYED FISH, *SEMOTILUS ATROMACULATUS*

Hart, Doudoroff, and
Greenbank, 1945

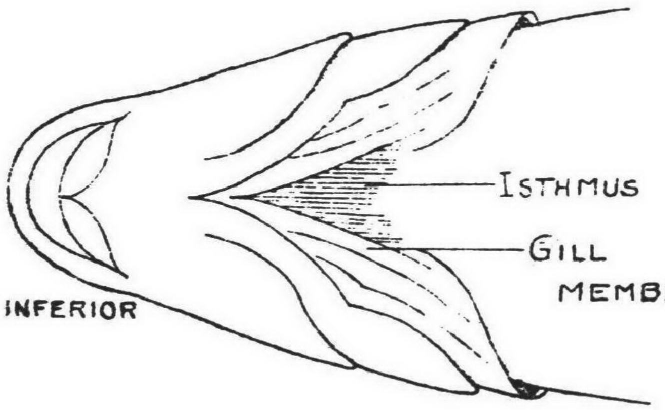
BI. AQ. pl. 9a. 6. 60



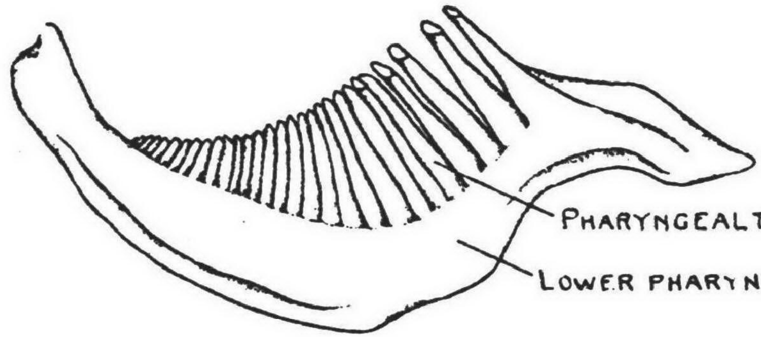
GILL MEMBRANES FREE OF THE ISTHMUS. AND SEPARATE



FORM OF PHARYNGEAL TEETH IN THE CYPRINIDAE.



GILL MEMBRANES SEPARATE AND CONNECTED TO THE ISTHMUS.



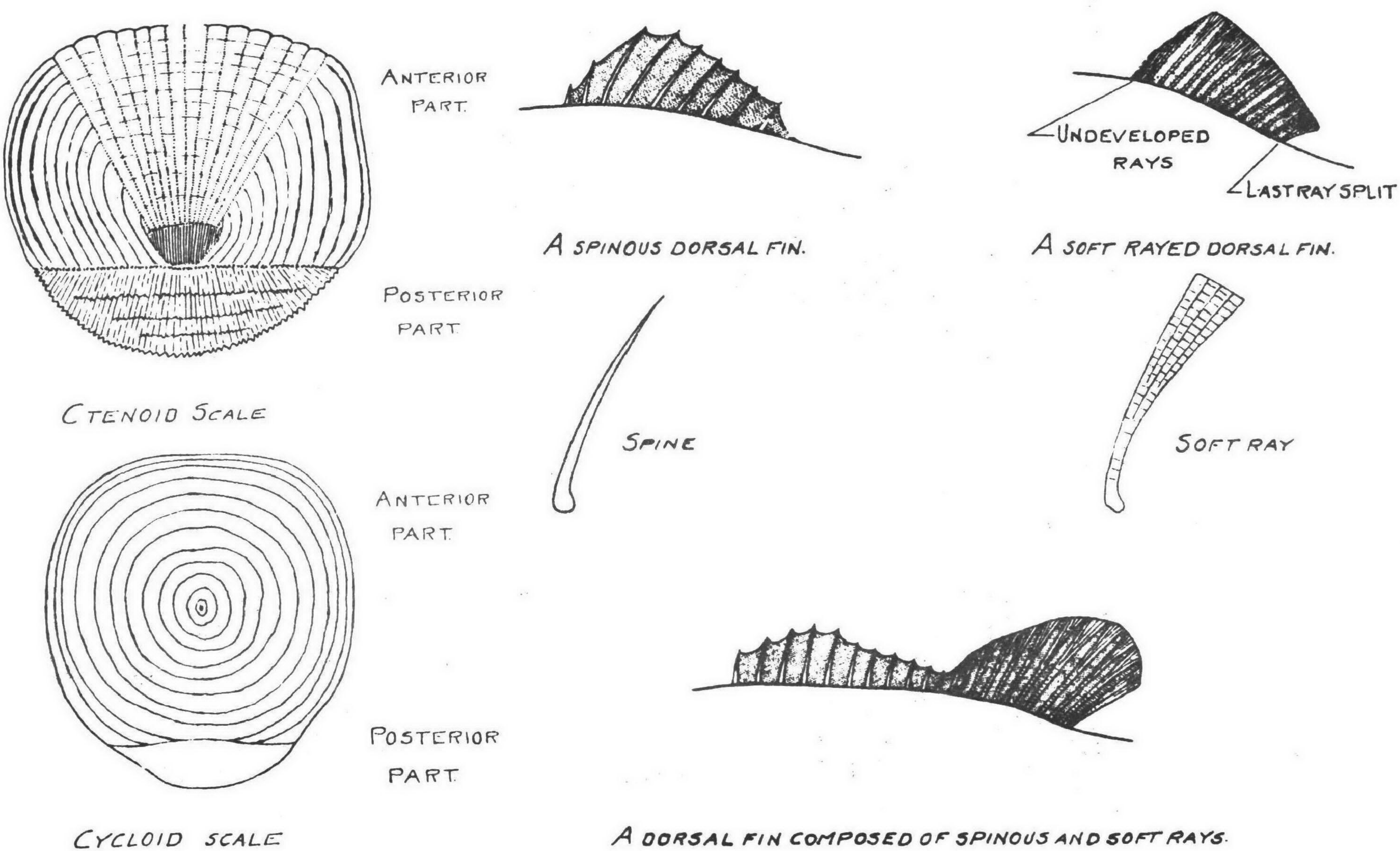
COMB-LIKE TEETH IN THE CATOSTOMIDAE.

Hart, Doudoroff, and Greenbank, 1945

BI. AQ. pl. 9c. 6. 60

This outline was prepared by H. W. Jackson

FIN AND SCALE STRUCTURES



Reproduced with permission; Hart. Doudoroff, and Greenbank, 1945.

CLASSIFICATION OF FISHES

A classification of fishes and lower vertebrates following recent researches reported in Romer 1946, Quarterly Review of Biology, Schultz 1948, Ways of Fishes and Berg 1940, Classification of Fishes. Those preceded by an asterisk are known from fossil forms only.

Phylum Chordata

Subphylum Hemichordata Acorn worms

Subphylum Tunicata (Urochordata) Ascidians Sea squirts

(Note the above two groups are not usually claimed nor studied by vertebrate zoologists).

Subphylum Acrania (without cranium)

Class Cephalochordata (Leptocardi) Amphioxi

Subphylum Craniata (with cranium) Equals Vertebrata of many authors

Superclass Agnatha (without jaws, with bone in part)

Contains four large well differentiated classes two of which are living and two known only from fossils. The latter are the oldest certain remains of vertebrates and are found in Ordovician rocks and are lumped usually under the term Ostracoderms.

Class Petromyzones (Marsipobranchii or Cyclostomata)

Lampreys. Bone lost.

Class Myxini Hagfishes. Bone lost.

*Class Pteraspides (Heterostraci) Lower Silurian to upper Devonian

*Class Cephalaspides (Osteostraci) Upper Silurian to upper Devonian

*Class Euphanerida (Jamoytius)

Superclass Gnathostomata (equals Pisces, with jaws)

*Class Placodermi (with bone)

Three subclasses (Berg gives them ranking of class) are usually recognized here. All are fossil.

*Subclass Pterichthyes (Antiarchi) Middle and upper Devonian. Pectoral appendages jointed.

*Subclass Coccostei (Arthrodira) Upper Silurian to upper Devonian (or to early Mississippian) Jointed neck, armored fishes.

*Subclass Acanthodii Spiny Sharks. Upper Silurian to lower Permian (acme in Lower Devonian).

Class Elasmobranchii (Chondrichthyes) Sharks and rays.

Bone lost, jaws advanced in structure. Upper Devonian to recent.

Class Holocephali. Chimaeras, elephant fishes, and rat fishes (given as subclass under Elasmobranchii by some authors) Cartilaginous, gill cover developed. Upper Devonian to recent.

Class Osteichthyes (Teleostomi of Berg) Bony Fishes.

Lower Devonian to recent.

Subclass Choanichthyes

Order Dipnoi Lungfishes Treated by Berg as a separate class placed just before Osteichthyes.

Middle (Lower?) Devonian to recent.

Order Crossopterygii Fringed fins. Lower Devonian to recent.

Latimeria from Southeast Africa

Subclass Actinopterygii Rayed Fins. Middle Devonian to recent.

Order Chrondrostei Polypteriforms to Acipenseriformes

Order Holostei Amiiformes, Lepidosteiformes to Pholiodophoriformes

Order Teleostei, Clupeiformes, etc.

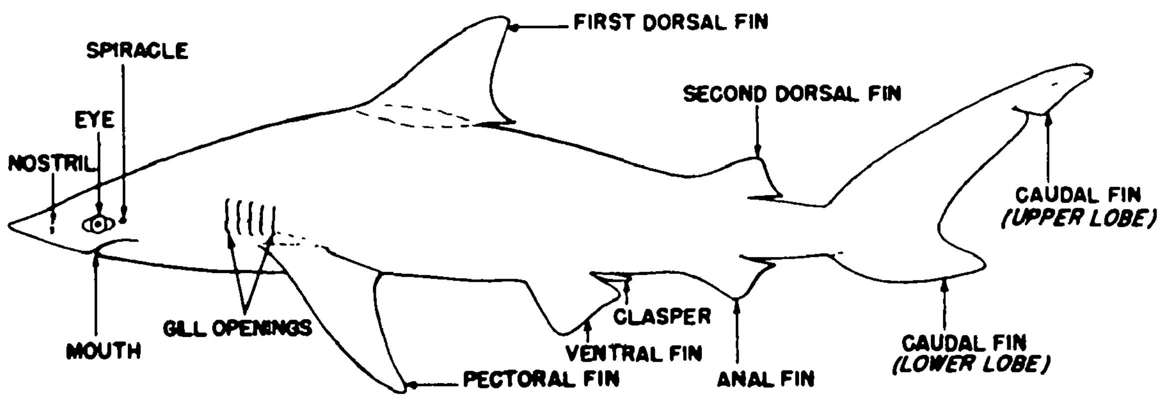


Figure 1. A TYPICAL SHARK SHOWING THE IMPORTANT EXTERNAL PARTS

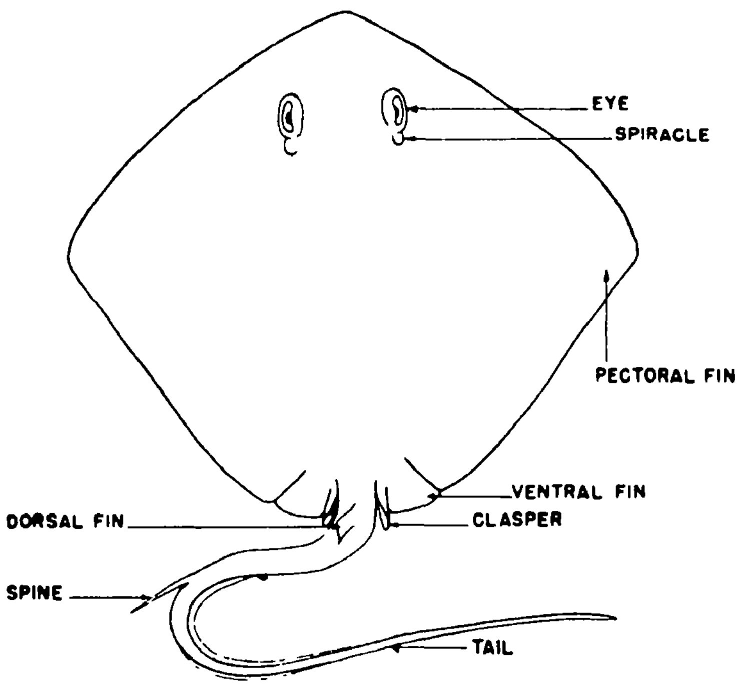


Figure 2. A TYPICAL RAY SHOWING THE IMPORTANT EXTERNAL PARTS

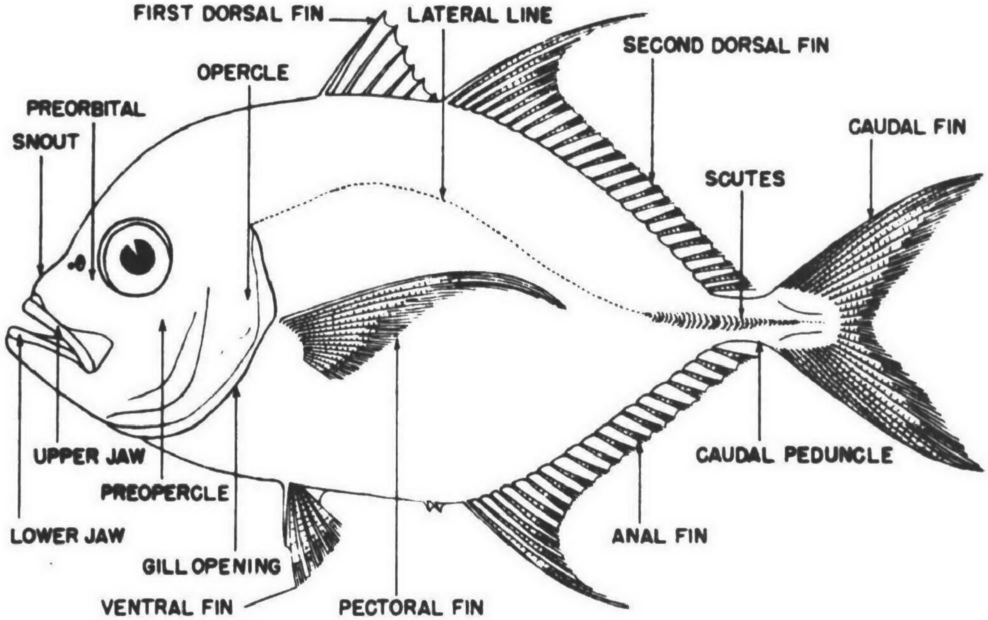


Figure 3. A TYPICAL BONY FISH SHOWING THE IMPORTANT EXTERNAL PARTS

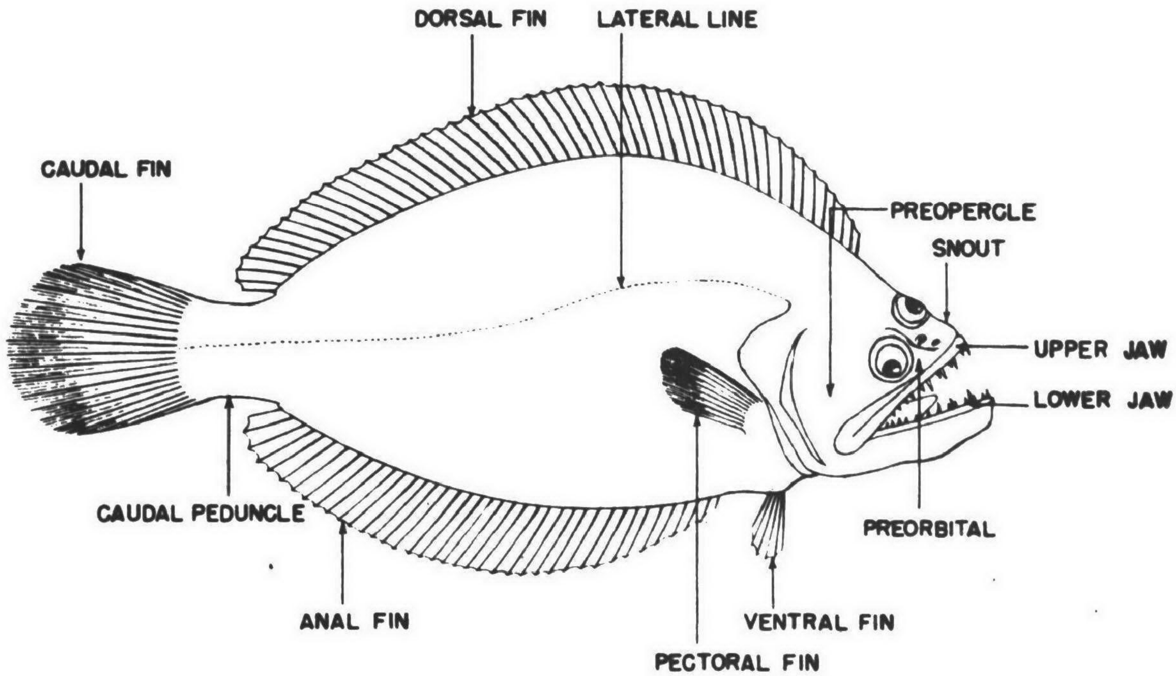


Figure 4. A FLATFISH, AN ABERRANT FORM OF BONY FISH

SECTION D

BACTERIA AND FUNGI: REDUCERS

This group of organisms as a group and as individuals exhibit a remarkable catholicity of taste. There is literally no known organic constituent of living things that cannot be used as food by at least one kind of bacterium or fungus. Clearly, this is essential to the continuing flow of carbon. Without this process carbon and energy would slowly but certainly accumulate in unused products of plant or animal metabolism. As will be shown in the following three outlines, the metabolsim of this group is marked by an enormous complexity and diversity. Underneath this complexity there is a fundamental oneness of mechanism shared by all living organisms. This unity in metabolism is further marked by the completing role of the reducers in nature as the indispensable feeders of the furnace of photosynthesis.

Contents of Section D

BIOLOGICAL REDUCERS

(The Role of Fungi in the Biodegradation of Organic Matter)

I INTRODUCTION

A Biological Classification System Based on Nutrition

Based on their fundamental nutritional mechanisms, all organisms can be segregated into three major groups, or "kingdoms" instead of the traditional two ("plant" vs "animal") kingdoms. These are

1 The green plants

The nutrition of the green plants is based on photosynthesis in which sunlight, carbon dioxide, water, and the chlorophyll of the green plant enter into a reaction (or series of *reactions) resulting in the synthesis of* sugars. From these fundamental building blocks the green plant synthesizes all the other organic compounds (utilizing other elements, notably nitrogen, phosphorus, sulfur, calcium, potassium, iron, and other elements in trace amounts).

2 The animals

Animals obtain their organic compounds, required for nutrition and growth, by ingestion of solid foods into the body structure. This food may be the bodies of green plants, the bodies of other animals, or it may be products, secretions, or excretions of either form of life.

3 The fungi

In a broad sense this includes both the filamentous fungi and the bacteria. These organisms obtain their food in solution, that is, they secrete extracellular enzymes into their environment, which break down complex organic matter sufficiently that it diffuses through the selectively permeable cell walls and cell membranes of the organism. Once within the cell structure, these dissolved organic materials are used for nutrition and growth in a manner comparable with that of the animals.

B Scope of this presentation

This discussion is concerned with the role of the fungi (including bacteria) in the natural self-purification processes occurring in the environment.

II NUTRITIONAL PROPERTIES OF THE FUNGI

A From species to species the fungi vary enormously in the details of their nutritional processes, and therefore, in the substances they are capable of using for food. In addition, they differ greatly from species to species in their environmental "preferences", resulting in a wide variety of species complexes which can be found from one aquatic habitat to another. Among the factors having great influence in selection of the species and species complexes which will appear in a given habitat may be cited

1 Oxygen, resulting in selectivity for strictly aerobic forms, or facultative forms.

2 pH, resulting in a tendency to select the forms having optimum growth and survival properties within a limited range of pH values. For example, most bacteria tend to grow best in pH levels at or near neutrality, while the filamentous fungi commonly have optimum growth properties

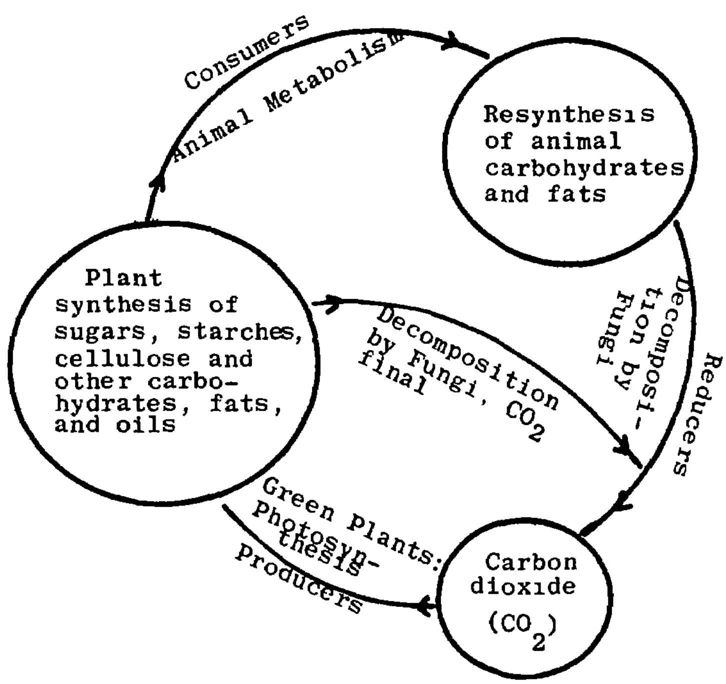
somewhat on the acid side of neutrality, say pH 5 to pH 7, or sometimes even lower.

3 Presence of certain organic growth requirements. some kinds of bacteria require external sources for complex organic substances that they require but cannot synthesize for themselves. Such organisms are, of course, highly limited and highly specific in the habitats in which they can compete successfully for the food.

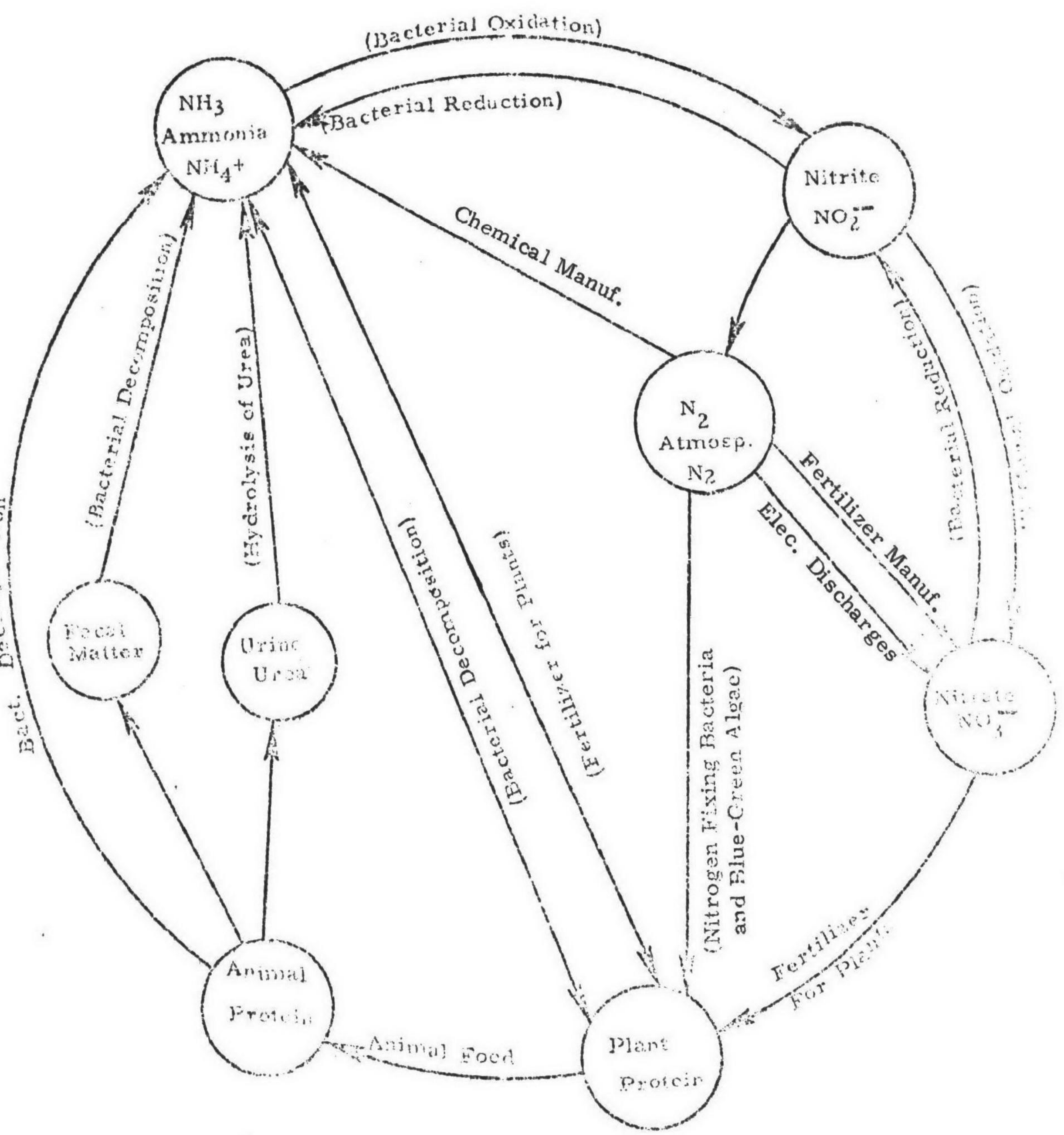
4 Nature of the substrate (food) available to the organisms, some kinds of bacteria are highly effective in utilizing carbohydrates (members of the Enterobacteriaceae, for example). There is a great deal of specificity from one kind of organism to another in the carbohydrates which can be metabolized. Others are not particularly active in using carbohydrates, but apparently are highly successful in using other forms of organic matter for food. (Pseumonadaceae, for example, are quite versatile in their nutritional capabilities.

B Cycles of Carbon, Nitrogen, and Phosphorus

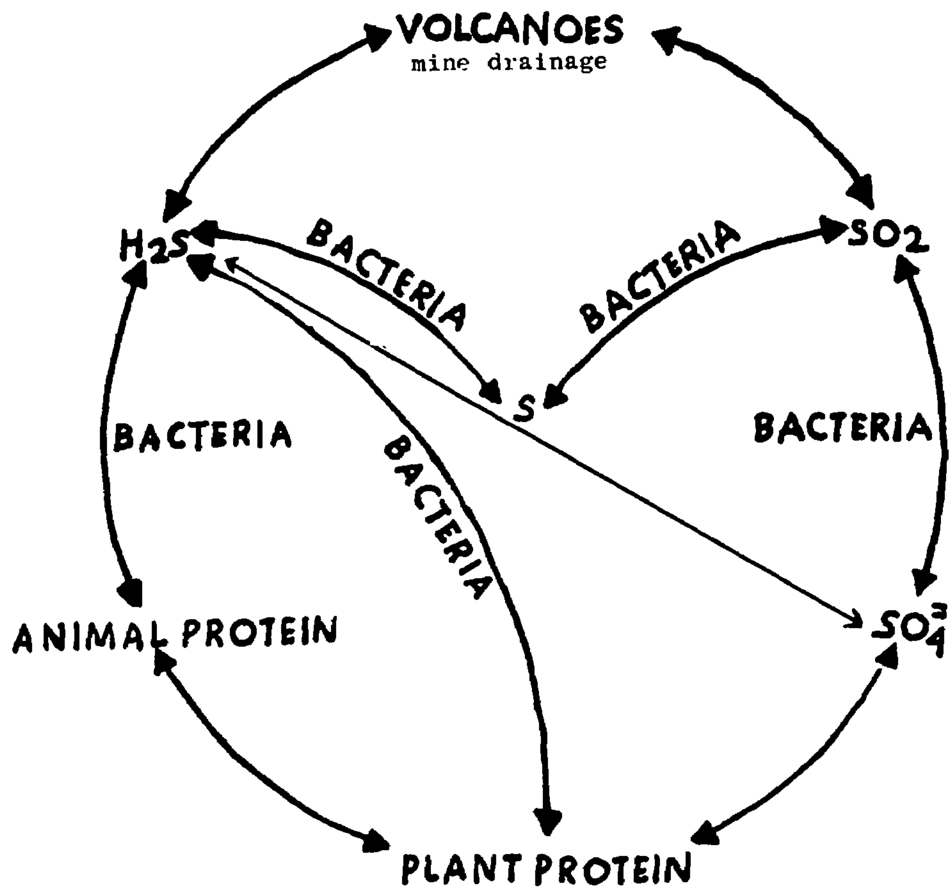
Incidental to their nutrition on which organic substances are used for food, the fungi play a vital role in the return to a mineral state of elements bound up in organic substances. The classical cycles of carbon, nitrogen, and phosphorus illustrate this role. These cycles may be represented diagramatically in a variety of degrees of complexity. The accompanying diagrams are intended to show the essential linkage of the fungi in completing the cycling of these substances, essential to life, which eventually would be bound up in the bodies of plants, animals, and their products, in the absence of a mechanism for returning the substances to a mineral state, with renewed availability to the green plant.



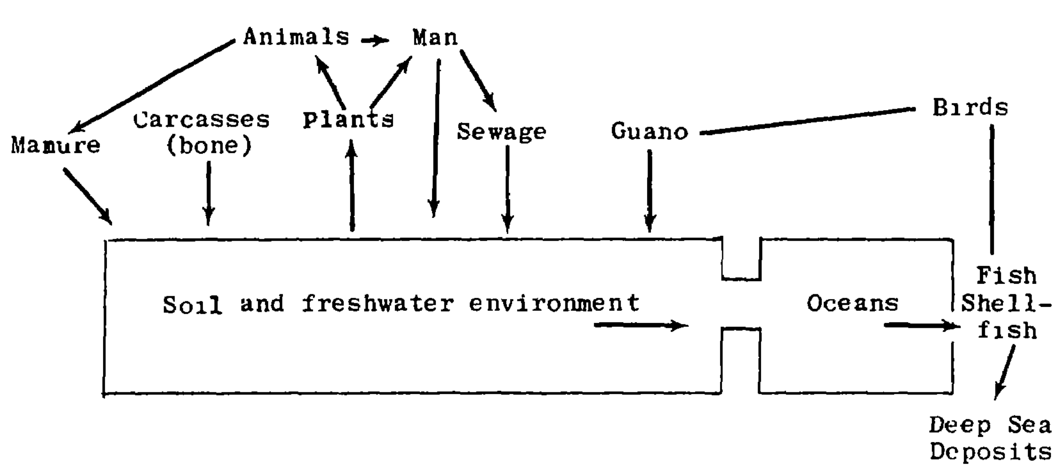
CARBON CYCLE (adapted after Hilliard, 1945)



NITROGEN CYCLE (after Allen, 1938)



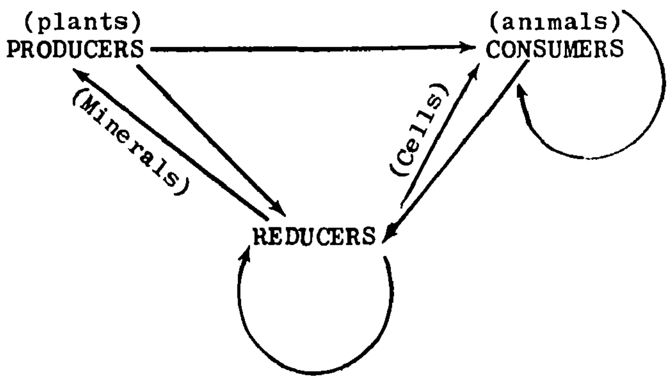
SULFUR CYCLE (after Salle, 1948)



PHOSPHORUS CYCLE (after Salle, 1948)

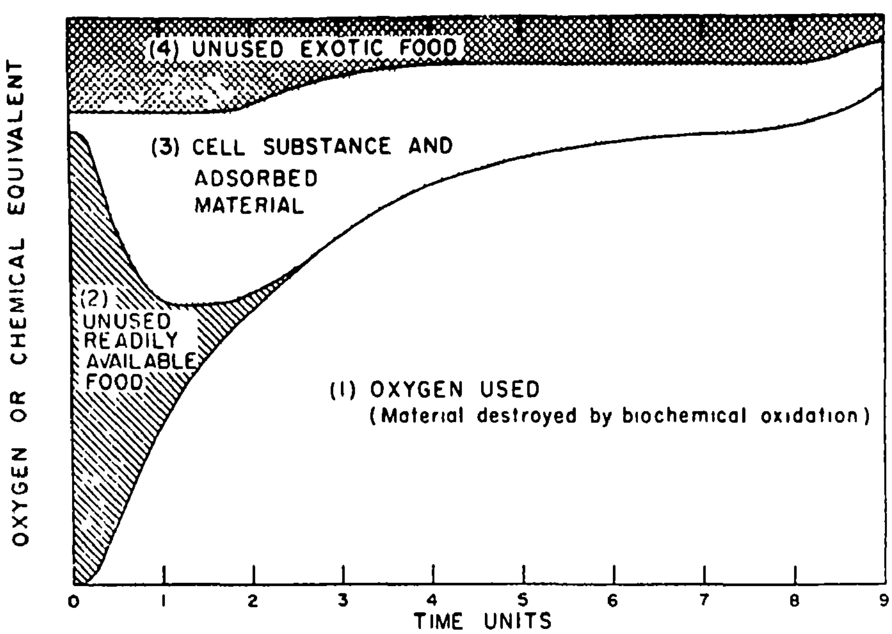
C Fate of Organic Matter Used for Nutrition

The complex and interrelated cycles illustrated above can be simplified along the following lines:



D In the process of reducing organic matter the fungi are playing the one "role" that appears basic with all forms of life to stay alive, to grow and reproduce. The utilization of the organic matter, then, does not result purely in the direct production of inorganic mineral substances. Much of this organic matter is utilized in the development of new cells of the bacteria and filamentous fungi.

This may be represented diagrammatically according to the figure showing sequence of metabolic change during oxidation of carbonaceous material. The figure represents "favorable conditions". As environmental conditions become "less favorable" the reserves of unused food persist for a greater period of time but the ultimate conversion to cell substance or to mineralized decomposition products ultimately will be the same.



SEQUENCE OF METABOLIC CHANGE DURING OXIDATION OF CARBONACEOUS MATERIAL (Favorable Conditions)

(Ettinger, 1951)

III ENVIRONMENTAL INTERRELATIONSHIPS

A Influence of Fungi on Their Environment

1 Dissolved oxygen

This topic is discussed at length elsewhere in this course manual. It is sufficient here to recall that the biochemical process of respiration, going on at all times in all living cells, results in utilization of oxygen (when available) as a hydrogen acceptor in the final stages of the respiratory reactions. When abundant food is present, and extremely large populations of bacteria develop rapidly, the oxygen in the aquatic habitat sometimes is used up more rapidly than it can be replenished from the air or from other sources. In such cases, extremes of oxygen depletion may occur, even including anaerobic situations.

2 pH

As a result of utilization of carbohydrates, organic acids with pH lower than neutrality (pH 7) may occur, with the effect of restricting the value of the habitat for some life forms, while tending to favor others (such as the filamentous fungi).

3 Mineralization

Return of sulfates, nitrates, phosphorus, and other elements to the aquatic environment as a result of bacterial activity has a "fertilizing" effect on such habitats. Often the result is prolific "blooms" of algae which in themselves may create a nuisance and may in turn bring about widely fluctuating levels of dissolved oxygen on a diurnal basis.

4 Antibiotic effects

Some kinds of bacteria and filamentous fungi excrete substances having antibiotic effects, killing or inactivating certain other forms of life. The Actinomyces, certain members of the genus Pseudomonas and certain forms of the filamentous fungus Penicillium are notable examples of antibiotic-producing members of the fungi.

B Influence of the Environment on Fungi

Environmental factors critical in selection of populations of bacteria and filamentous fungi which may be expected in an aquatic environment include, but are not necessarily limited to:

1 pH

2 Temperature

3 Salinity

4 Other organisms, especially predators or antibiotic-producers

5 Available nutrient materials and special growth factors if required

IV POLLUTION CONTROL SIGNIFICANCE

A In the aquatic environment the bacteria and filamentous fungi utilize organic substrates, thereby carrying on functions regarded desirable to humans and generally lumped as "natural self purification". When the amount of organic matter in the environmental water is not excessive and properties of the water are suitable for growth of these organisms, mineralization goes on without sharp buildup of carbon, nitrogen, phosphorus, and other elements. Further, oxygen required in respiratory processes does not significantly deplete the level dissolved in the water, and is readily replaced in solution from the overlying air. Nuisance conditions do not develop here. On the other hand, in situations where the concentration of organic matter is great, another set of conditions can develop with excessive buildup of minerals in solution, depletion of oxygen, and violent changes in the biota capable of surviving in such habitats.

B In wastewater treatment plants, engineers make use of the properties of the fungi in returning organic matter to circulation through development of structures and processes designed to utilize the capabilities of these organisms under controlled conditions in order to minimize the amount of organic material discharged to the aquatic habitat. Trickling filters, activated sludge treatment systems, sludge digestion systems, and septic tanks with associated tile fields all make use of biological processes for such processes. Bacterial processes have been regarded as predominant in such systems. Increasing evidence is appearing that filamentous fungi have a significant role to play, particularly in trickling filters.

REFERENCES

Any one of the many basic text books available on bacteriology.

1 Ettinger, M.B. Biochemical Oxidation Characteristics of Stream-Pollutant Organics. Industrial and Engineering Chemistry, 48 256. February 1956.

2 Kabler, P.W. Selection and Adaptation of Microorganisms in Waste Treatment. American Journal of Public Health, 50 215. February 1960.

This outline was prepared by Harold L. Jeter, Director, National Training Center, WPO, Cincinnati, OH 45268.

BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera Streptococcus, Lactobacillus, Staphylococcus, Proteus, Pseudomonas, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following

a Bacteria Species of Salmonella, Shigella, Leptospira, Brucella, Mycobacterium, and Vibrio comma.

b Viruses. A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: Endamoeba histolytica

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows·

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of pollution

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should·

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --

a Its density should have some direct relationship to the degree of fecal pollution.

b It should have greater survival time in water than enteric pathogens, throughout its course of natural disappearance from the water body.

c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.

d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality Surveys

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the Sphaerotilus group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as Pseudomonas, Achromobacter, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms _Bacterium coli-commune_ and _B. lactis aerogenes_. In 1895, another bacteriologist, Migula, renamed _B. coli commune_ as _Escherichia coli_, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (13th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C "

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above

a _Escherichia coli, E. aurescens, E. freundii, E. intermedia_

b _Aerobacter aerogenes, A. cloacae_

c Biochemical intermediates between the genera _Escherichia_ and _Aerobacter_

* The above terminology is in accordance with the current editions of Standard Methods and Bergey's Manual of Determinative Bacteriology and will be consistent throughout this manual until these sources are modified.

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical _E. coli_ and closely related strains are of fecal origin while _A. aerogenes_ and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here·

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by A. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). A. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas A. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2, 3, butylene-glycol, as a result of glucose fermentation in the presence of peptone. A. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to $2\frac{1}{2}$ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of A. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2°C are the current recommended medium and method of choice. While the "EC" terminology of the medium suggests "E. coli" the worker should not regard this as a specific procedure for isolation of E. coli.

c A similar medium, Boric Acid Lactose Broth, has been developed by Levine and his associates This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevated temperature tests require incubation in a water bath Standard Methods 13th Ed. requires this temperature to be 44.5 ± 0.2°C. Various workers have urged use of temperatures ranging between 43.0°C and 46.0°C. Most of these recommendations have provided a tolerance of ± 0.5°C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2°C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range has been instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of E. coli would have a code ++--, and a typical A. aerogenes culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References of this outline are suggested routes of entry for workers seeking to explore this topic.

B Studies on Coliform Distribution

1 Since 1960 numerous workers have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

a Rigid uniformity of laboratory methods have been applied throughout the series of studies

b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

Groupings of Coliforms into Fecal, Nonfecal and Intermediate Groups

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
E. coli, Variety I	+	+	-	-
Variety II	-	+	-	-
E. freundii (Intermediates)				
Variety I	-	+	-	±
Variety II	+	+	-	+
A. aerogenes				
Variety I	-	-	+	±
Variety II	±	-	+	+

c A wider variety of environmental and biological sources is being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year. It is believed that no more representative series of studies has been made or is in progress.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human feces also includes numbers of A. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes, on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments.

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Variety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin

b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers

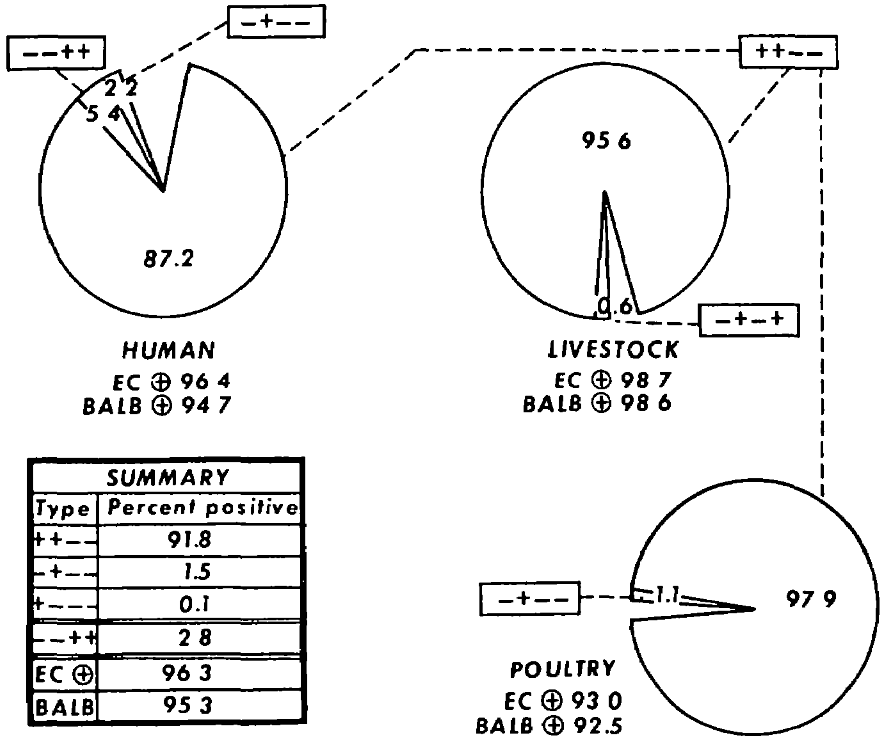
c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMViC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMViC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10. 6	134	12. 4	131	5. 6	536	80. 6	3932	87. 2	2237	95. 6	1857	97. 9
--++	237	19. 7	113	10. 4	443	18. 8	13	2. 0	245	5. 4	0	<0. 1	1	0. 1
-+--	23	1. 9	0	<0. 1	78	3. 3	1	0. 2	99	2. 2	14	0. 6	20	1. 1
+++-	2	0. 2	0	<0. 1	7	0. 3	0	<0. 1	106	2. 4	59	2. 5	0	<0. 1
-+-+	168	14. 0	332	30. 6	1131	48. 1	87	13. 0	50	1. 1	1	<0. 1	5	0. 3
++-+	116	9. 6	118	10. 9	87	3. 7	22	3. 3	35	0. 8	27	1. 2	11	0. 6
-+++	32	2. 7	28	2. 6	181	7. 7	5	0. 7	21	0. 5	0	<0. 1	0	<0. 1
++++	291	24. 2	254	23. 4	159	6. 8	0	<0 1	6	0. 1	0	<0. 1	0	<0. 1
+-++	88	7. 3	46	4. 2	67	2. 9	0	<0. 1	14	0. 2	0	<0. 1	0	<0. 1
---+	87	7. 2	42	3. 9	4	0. 2	1	0. 2	2	<0. 1	0	<0. 1	0	<0. 1
-++-	5	0. 4	0	<0. 1	1	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1
--+-	19	1. 6	0	<0. 1	53	2. 3	0	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1
+-+-	2	0. 2	0	<0. 1	6	0. 3	0	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1
+--+	5	0. 4	8	0. 7	0	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1	0	<0. 1
+---	0	<0. 1	9	0. 8	0	<0. 1	0	<0. 1	2	<0. 1	0	<0. 1	2	<0. 1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14. 1*		14. 9*		9. 2		82. 9		96. 4		98. 7		93. 0	

*120 of these were ++--, 15 --++, 11 -+--

*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++--	91.8
-+--	1.5
+---	0.1
--++	2 8
EC ⊕	96 3
BALB	95 3

FIGURE 1

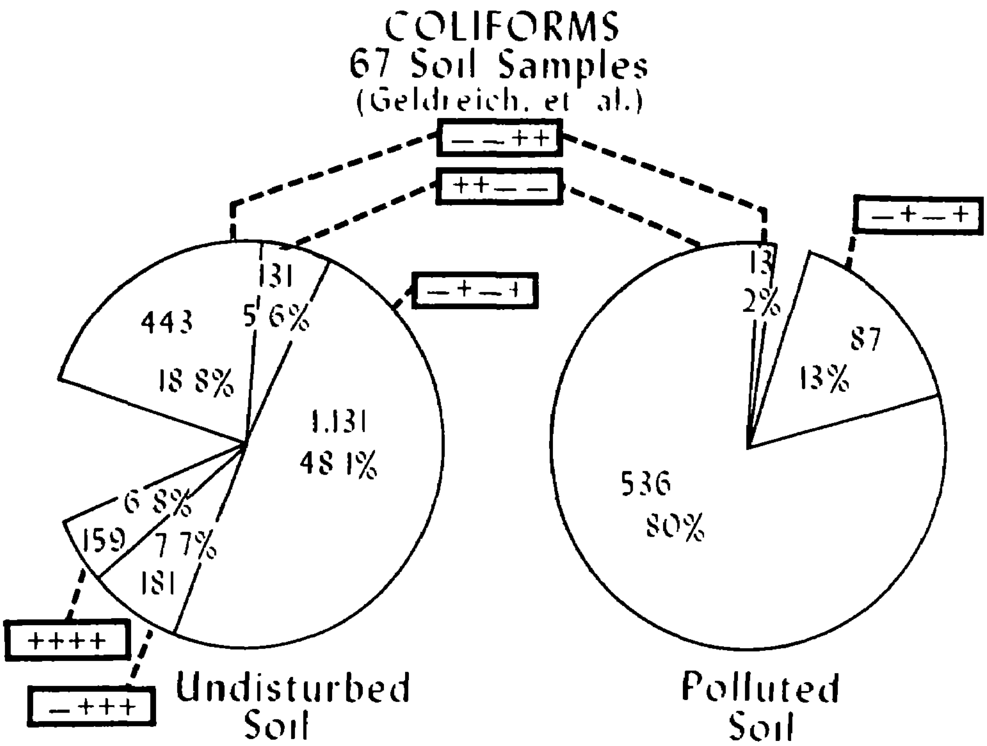


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMViC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965, Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil. Unpolluted	Soil. Polluted	Vegetation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5.1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85.1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is regarded as the method of choice for differentiation between coliforms of probable direct fecal origin and those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the A. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of non-coliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation These organisms are E coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c At this time, evaluations are underway regarding the survival of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (13th ed., 1971), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods introduced the standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been carried to the 13th Edition.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I INTRODUCTION

Investigations regarding streptococci progressed from the streptococci of medical concern to those which were distributed in differing environmental conditions which, again, related to the welfare of man. The streptococci were originally reported by Laws and Andrews (1894), and Houston (1899, 1900) considered those streptococci, which we now call "fecal streptococci," as ... "indicative of dangerous pollution, since they are readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination.

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, significant body of technical literature has appeared.

This outline will consider the findings of various investigators regarding the fecal streptococci and the significance of discharges of these organisms into the aquatic environment.

II FECAL MATERIALS

A Definition

The terms "enterococci," "fecal streptococci," "Group D streptococci," "Streptococcus fecalis," and even "streptococci" have been used in a loose and interchangeable manner to indicate the streptococci present in the enteric tract of warm-blooded animals or of the fresh fecal material excreted therefrom.

Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

For the proposes of this outline, and in line with the consensus of most water microbiologists in this country, the definition of the fecal streptococci is·

> "The group composed of Group D species consistently present in significant numbers in fresh fecal excreta of warm-blooded animals, which includes all of the enterococcus group in addition to other groups of streptococci."

B Species Isolated

1 Findings

a Human feces

Examination of human fecal specimens yields a high percentage of the enterococcus group and usually demonstration of the S. salivarius which is generally considered a member of the human throat flora and to be surviving in human fecal materials rather than actively multiplying in the enteric tract. Also present would be a small percentage of variants or biotypes of the enterococcus group.

b Nonhuman Feces

1) Fecal material which are from nonhuman or not from fowl will yield high percentages of the S. bovis and/or S. equinus organisms with a concomitantly reduced percentage of the enterococcus group.

2) Fowl excreta

Excrement from fowl characteristically yields a large percentage of enterococcal biotypes as well as a significant percentage of enterococcus group.

2 Significance

Species associations with particular animal hosts is an established fact and leads to the important laboratory technique of partition counting of colonies from the membrane filter or pour agar plates in order to establish or confirm the source of excretal pollution in certain aquatic investigations.

It is important to realize that a suitable medium is necessary in order to allow all of the streptococci which we consider to be fecal streptococci to grow in order to give credence to the derived opinions. Use of liquid growth media into which direct inoculations from the sample are made have not proven to be successful for partition counting due to the differing growth rates of the various species of streptococci altering the original percentage relationships. Due to the limited survival capabilities of some of the fecal streptococci it is necessary to sample fresh fecal material or water samples in close proximity to the pollution source especially when multiple sources are contributing to a reach of water. Also the pH range must be within the range of 4.0-9.0.

III FECAL STREPTOCOCCI IN THE AQUATIC ENVIRONMENT

A General

From the foregoing it is apparent that the preponderant human fecal streptococci is composed of the enterococcus group and, as this is the case, several media presently available which will detect only the enterococcal group will be suitable for use with aquatic samples which are known to be contaminated or potentially contaminated with purely domestic (human) wastes. On the other hand, when it is known or suspected that other-than-human wastes have potential egress to the aquatic environment under investigation, it is necessary to utilize those media which are capable of quantitating the whole of the fecal streptococci group.

B Stormwaters and Combined Sewers

1 General

Storm sewers are a series of pipes and conduits which receive surface runoffs from the action of rainstorms and do not include sewage which are borne by a system of sanitary sewers. Combined sewers receive both the runoff rains as well as the water borne wastes of the sanitary system. Both of these types of runoffs can be discharged to the aquatic environment and the usual instance where this occurs, with respect to the combined sewers, is when the amount of flow is in excess of the amounts capable of being treated during the high flow conditions. Both of these discharge forms have been found to usually contain large quantities of fecal streptococci and in numbers which generally are larger than that of the fecal coliform indicator organism. Stormwaters can be concluded to represent a typical stream environment with respect to the presence of chemical constituents and show a wide range of electrolytes which at times simulate that of irrigation waters.

2 Bacteriological Findings

Table 1 represents, in a modified form, some of the findings of Geldreich and Kenner (1969) with respect to the densities of fecal streptococci when considering Domestic sewage in contrast to Stormwaters:

Table 1

DISTRIBUTION OF FECAL STREPTOCOCCI IN DOMESTIC SEWAGES AND STORMWATER RUNOFFS

Water Source	Fecal Streptococci per 100 ml median values	Ratio FC/FS
Domestic Sewage		
Preston, ID	64,000	5.3
Fargo, ND	290,000	4.5
Moorehead, MN	330,000	4.9
Cincinnati, OH	2,470,000	4.4
Lawrence, MA	4,500,000	4.0
Monroe, MI	700,000	27.9
Denver, CO	2,900,000	16.9
Stormwater		
Business District	51,000	0.26
Residential	150,000	0.04
Rural	58,000	0.05

The Ratio FC/FS is that of the Fecal coliform and Fecal streptococci and it will be noted that in each case, when considering the Domestic Sewage, it is 4.0 or greater while it is less than 0.7 for stormwaters. The use of this ratio is useful to identify the source of pollution as

Table 2. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

being human or nonhuman warm-blooded animal polluted. When the ratio is greater than 4.0 it is considered to be human waste contaminated while a ratio of less than 0.7 is considered to be nonhuman. It is evident that the storm-waters have been primarily polluted by excreta of rats and other rodents and possibly domestic and/or farm animals.

Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 2 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators.

C Surface Waters

In general, the occurrence of fecal streptococci indicates fecal pollution and its absence indicates that little or no warm-blooded fecal contribution. In studies of remote surface waters the fecal streptococci are infrequently isolated and occurrences of small numbers can be attributed to wild life and/or snow melts and resultant drainage flows.

Various examples of fecal streptococcal occurrences are shown in Table 3 in relation to surface waters of widely varying quality. (Geldreich and Kenner 1969)

Table 3

INDICATOR ORGANISMS IN SURFACE WATERS

Water Source	Densities/100 ml Fecal coliform	Fecal streptococci
Prairie Watersheds		
Cherry Creek, WY	90	83
Saline River, KS	95	180
Cub River, ID	110	160
Clear Creek, CO	170	110
Recreational Waters		
Lake Mead	2	444
Lake Moovalaya	9	170
Colorado River	4	256
Whitman River	32	88
Merrimack River	100	96
Public Water Intakes		
Missouri River (1959)		
Mile 470.5	11,500	39,500
Mile 434.5	22,000	79,000
Mile 408.8	14,000	59,000

IV FECAL STREPTOCOCCI: ADVANTAGES AND LIMITATIONS

A General

Serious studies concerning the streptococci were instituted when it became apparent that they were the agents responsible or suspected for a wide variety of human diseases. Natural priority then focused itself to the taxonomy of these organisms and this study is still causing consternation as more and more microbiological techniques have been brought to bear on these questions. The sanitary microbiologist is concerned with those streptococci which inhabit the enteric tract of warm-blooded animals, their detection, and utilization in developing a criterium for water quality standards.

Kabler (1962) discussed the slow acceptance of the fecal streptococci as indicators of pollution resulting from:

1 Multiplicity and difficulty of laboratory procedures

2 Poor agreement between methods of quantitative enumeration

3 Lack of systematic studies of . . . .

a sources

b survival, and

c interpretations, and

4 Undue attention to the S. faecalis group.

Increased attention to the fecal streptococci, especially during the last decade, have clarified many of the earlier cloudy issues and have elevated the stature of these organisms as indicators of pollution. Court precedents establishing legal status and recommendations of various technical advisory boards have placed the fecal coliform group in a position of primacy in many water quality applications. The fecal streptococci have evolved from a position of a theoretically useful indicator to one which was ancillary to the coliforms to one which was useful when discrepancies or questions evolved as to the validity of the coliform data to one where an equality status was achieved in certain applications. In the future it is anticipated that, for certain applications, the fecal streptococci will achieve a position of primacy for useful data, and, as indicated by Litsky (1955) "be taken out of the realm of step-children and given their legitimate place in the field of santiary bacteriology as indicators of sewage pollution."

B Advantages and Limitations

1 Survival

In general, the fecal streptococci have been observed to have a more limited survival time in the aquatic environment when compared to the coliform group. They are rivaled in this respect only by the fecal coliforms. Except for cases of persistence in waters of high electrolytic content, as may be common to irrigation waters, the fecal streptococci have not been observed to multiply in polluted waters as may sometimes be observed for some of the coliforms. Fecal streptococci usually require a greater abundance of nutrients for survival as compared to the coliforms and the coliforms are more dependent upon the oxygen tension in the waterbody. In a number of situations it was concluded that the fecal streptococci reached an extinction point more rapidly in warmer waters while the reverse was true in the colder situations as the coliforms now were totally eliminated sooner.

2 Resistance to Disinfection

In artificial pools the source of contamination by the bathers is usually limited to throat and skin flora and thus increasing attention has been paid to indicators other than those traditionally from the enteric tract. Thus, one of the organisms considered to be a fecal streptococci, namely, S. salivarius, can be a more reliable indicator when detected along with the other fecal streptococci especially since studies have confirmed the greater resistance of the fecal streptococci to chlorination. This greater resistance to chlorination, when compared to the fecal coliforms, is important since the dieoff curve differences are insignificant when the curves of the fecal coliforms are compared to various Gram negative pathogenic bacteria which reduces their effectiveness as indicators.

3 Ubiquitous Strains

Among the fecal streptococcus are two organisms, one a biotype and the other a variety of the S. faecalis, which, being ubiquitous (omnipresent) have limited sanitary significance. The biotype, or atypical, S. faecalis is characterized by its ability to hydrolyze starch while the varietal form, liquefaciens, is nonbeta haemolytic and capable of liquefying gelatin. Quantitation of these organisms in anomalous conditions is due to their capability of survival in soil or high electrolytic waters and in waters with a temperature of less than 12 Degrees C.

Samples have been encountered which have been devoid of fecal coliforms and yet contain a substantial number of "fecal streptococci" of which these ubiquitous strains constitute the majority or all of the isolations when analyzed biochemically.

V STANDARDS AND CRITERIA

Acceptance and utilization of Total Coliform criteria, which must now be considered a pioneering effort, has largely been supplanted in concept and in fact by the fecal coliforms in establishing standards for recreational waters.

The first significant approach to the utilization of the fecal streptococci as a criterium for recreational water standards occurred in 1966 when a technical committee recommended the utilization of the fecal streptococci with the total coliforms as criteria for standards pertaining to the Calumet River and lower Lake Michigan waters. Several sets of criteria were established to fit the intended uses for this area. The use of the fecal streptococci as a criterium is indicated to be tentative pending the accumulation of existing densities and could be modified in future standards.

With the existing state-of-the-art knowledge of the presence of the fecal streptococci in waters containing low numbers of fecal coliforms it is difficult to establish a specific fecal streptococcus density limit of below 100 organisms/100 ml when used alone or in conjunction with the total coliforms.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

a Detection of changes in the bacterial composition of a water source

b Process control procedures in treatment plant operations

c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because.

a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

b No information of any kind is given about the species of bacteria cultivated.

c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

a There is no total bacterial count standard for any of the following

Interstate Quarantine Drinking Water Standards

PHS regulations for water potability (as shown in "Standard Methods" Public Health Service Drinking Water Standards of 1962.)

b The most widely used current application of total bacterial counts in water bacteriology today is in water treatment plants, where some workers use standard plate counts for process control and for determination of the bacterial quality of distribution systems and equipment.

c Total bacterial counts are not used in PHS water quality studies, though extensively used until the 1940's.

B Spore-Forming Bacteria (Clostridium perfringens, or C. welchii)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of C. perfringens.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include Salmonella sp, Shigella sp, Vibrio comma, Mycobacterium sp, Pasteurella sp, Leptospira sp, and others.

2 Merits of direct tests

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism, thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and investigated to some extent. Mention can be made, however, of consideration of tests for the following.

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Serological procedures for detection of coliforms and other indicators, a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

3 Tests for Pseudomonas aeruginosa

4 Tests for viruses, which may persist in waters even longer than members of the coliform group.

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FUNGI AND THE "SEWAGE FUNGUS" COMMUNITY

I INTRODUCTION

A Description

Fungi are heterotrophic achylorophyllous plant-like organisms which possess true nuclei with nuclear membranes and nucleoli. Dependent upon the species and in some instances the environmental conditions, the body of the fungus, the thallus, varies from a microscopic single cell to an extensive plasmodium or mycelium. Numerous forms produce macroscopic fruiting bodies.

B Life Cycle

The life cycles of fungi vary from simple to complex and may include sexual and asexual stages with varying spore types as the reproductive units.

C Classification

Traditionally, true fungi are classified within the Division Eumycotina of the Phylum Mycota of the plant kingdom. Some authorities consider the fungi an essentially monophyletic group distinct from the classical plant and animal kingdoms.

II ACTIVITY

In general, fungi possess broad enzymatic capacities. Various species are able to actively degrade such compounds as complex polysaccharides (e.g., cellulose, chitin, and glycogen), proteins (casein, albumin, keratin), hydrocarbons (kerosene) and pesticides. Most species possess an oxidative or microaerophilic metabolism, but anaerobic catabolism is not uncommon. A few species show anaerobic metabolism and growth.

III ECOLOGY

A Distribution

Fungi are ubiquitous in nature and members of all classes may occur in large numbers in aquatic habitats. Sparrow (1968) has briefly reviewed the ecology of fungi in freshwaters with particular emphasis on the zoosporic phycomycetes. The occurrence and ecology of fungi in marine and estuarine waters has been examined recently by a number of investigators (Johnson and Sparrow, 1961, Johnson, 1968, Myers, 1968, van Uden and Fell, 1968).

B Relation to Pollution

Wm. Bridge Cooke, in a series of investigations (Cooke, 1965), has established that fungi other than phycomycetes occur in high numbers in sewage and polluted waters. His reports on organic pollution of streams (Cooke, 1961, 1967) show that the variety of the Deuteromycete flora is decreased at the immediate sites of pollution, but dramatically increased downstream from these regions.

Yeasts, in particular, have been found in large numbers in organically enriched waters (Cooke, et al., 1960, Cooke and Matsuura, 1963, Cooke, 1965b; Ahearn, et al., 1968). Certain yeasts are of special interest due to their potential use as "indicator" organisms and their ability to degrade or utilize proteins, various hydrocarbons, straight and branch chained alkyl-benzene sulfonates, fats, metaphosphates, and wood sugars.

C "Sewage Fungus" Community (Plate I)

A few microorganisms have long been termed "sewage fungi." The most common microorganisms included in this group are the iron bacterium Sphaerotilus natans and the phycomycete Leptomitus lacteus.

1 Sphaerotilus natans is not a fungus, rather it is a sheath bacterium of the order chlamydobacteriales. This polymorphic bacterium occurs commonly in organically enriched streams where it may produce extensive slimes.

a Morphology

Characteristically, S. natans forms chains of rod shaped cells (1.1 - 2.0μ x 2.5 - 17μ) within a clear sheath or trichome composed of a protein-polysaccharidae-lipid complex. The rod cells are frequently motile upon release from the sheath; the flagella are lophotrichous. Occasionally two rows of cells may be present in a single sheath. Single trichomes may be several mm in length and bent at various angles. Empty sheaths, appearing like thin cellophane straws, may be present

b Attached growths

The trichomes are cemented at one end to solid substrata such as stone or metal, and their cross attachment and bending gives a superficial similarity to true fungal hyphae. The ability to attach firmly to solid substrates gives S. natans a selective advantage in the population of flowing streams. For more thorough reviews of S. natans see Prigsheim (1949) and Stokes (1954).

2 Leptomitus lacteus also produces extensive slimes and fouling flocs in fresh waters. This species forms thalli typified by regular constrictions.

a Morphology

Cellulin plugs may be present near the constrictions and there may be numerous granules in the cytoplasm. The basal cell of the thallus may possess rhizoids.

b Reproduction

The segments delimited by the partial constrictions are converted basipetally to sporangia. The zoospores are diplanetic (i.e., dimorphic) and each possesses one whiplash and one tinsel flagellum. No sexual stage has been demonstrated for this species.

c Distribution

For further information on the distribution and systematics of L. lacteus see Sparrow (1960), Yerkes (1966) and Emerson and Weston (1967). Both S. natans and L. lacteus appear to thrive in organically enriched cold waters (5^{o}-$22^{o}C$) and both seem incapable of extensive growth at temperatures of about $30^{o}C$.

d Gross morphology

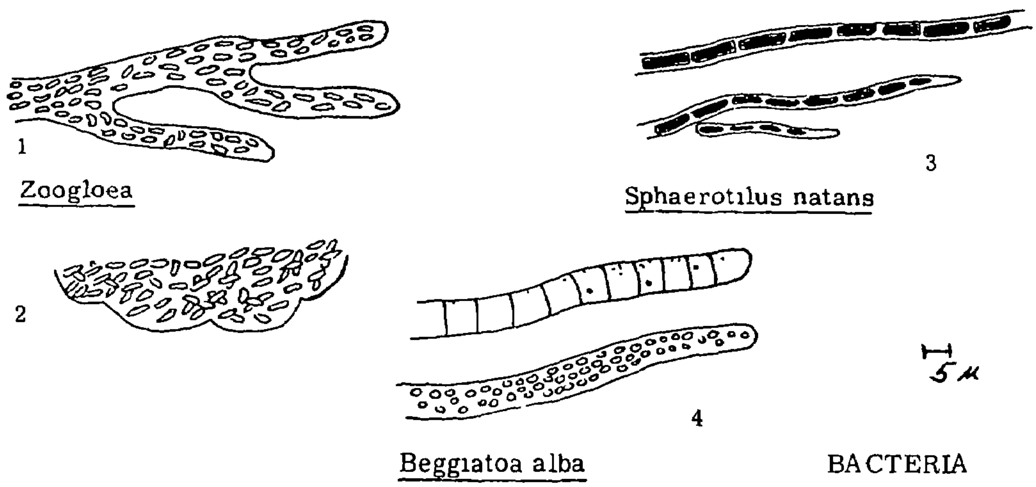
Their metabolism is oxidative and growth of both species may appear as reddish brown flocs or stringy slimes of 30 cm or more in length.

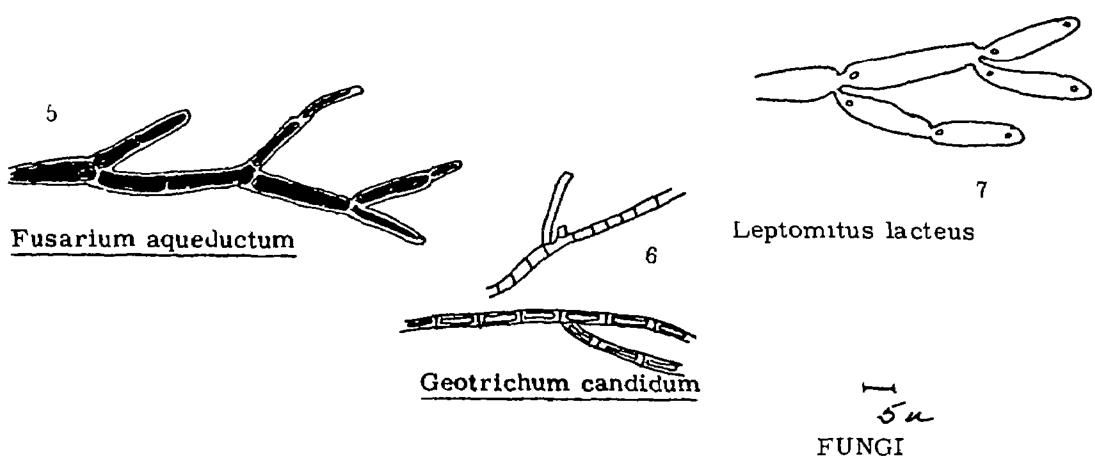
e Nutritive requirements

Sphaerotilus natans is able to utilize a wide variety of organic compounds, whereas L. lacteus does not assimilate simple

PLATE I

"SEWAGE FUNGUS" COMMUNITY OR "SLIME GROWTHS"
(Attached "filamentous" and slime growths)





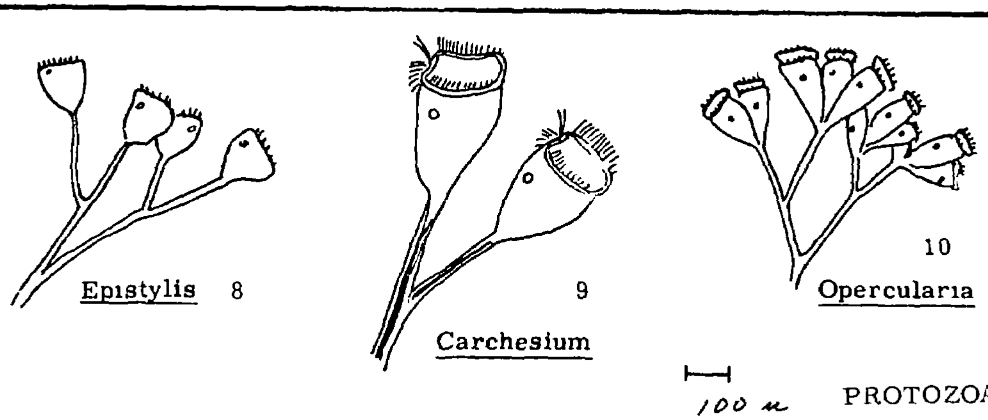


PLATE II

REPRESENTATIVE FUNGI

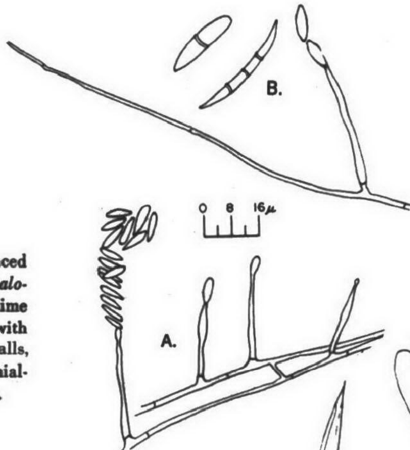


Figure 1

Fusarium aquaeductuum
(Radlmacher and Rabenhorst) Saccardo

Microconidia (A) produced from phialides as in *Cephalosporium*, remaining in slime balls. Macroconidia (B), with one to several cross walls, produced from collared phialides. Drawn from culture.

Figure 2

***Leptomitus lacteus* (Roth) Agardh**

Cells of the hyphae showing constrictions with cellulin plugs. In one cell large zoospores have been delimited. Redrawn from Coker, 1923.

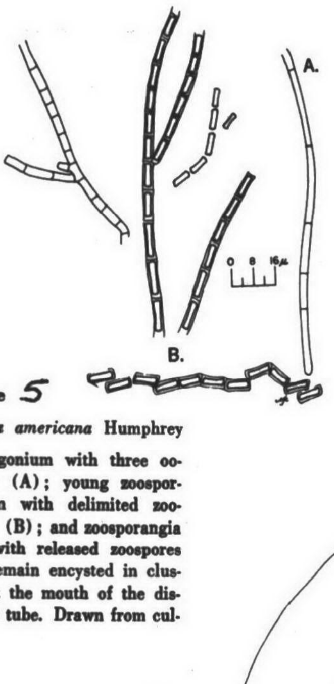


Figure 3

Geotrichum candidum
Link ex Persoon

Mycelium with short cells and arthrospores. Young hypha (A); and mature arthrospores (B). Drawn from culture.

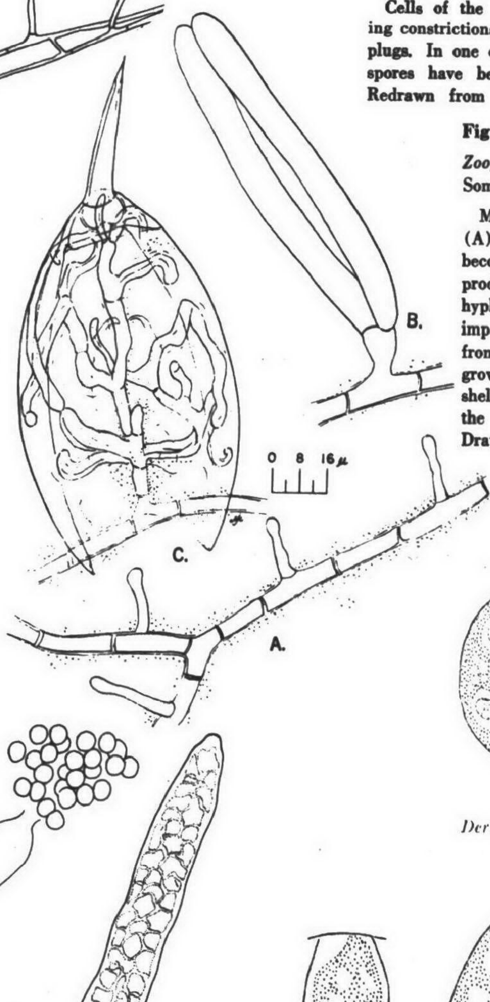


Figure 4

Zoophagus insidians
Sommerstorff

Mycelium with hyphal pegs (A) on which rotifers will become impaled; gemmae (B) produced as conidia on short hyphal branches; and rotifer impaled on hyphal peg (C) from which hyphae have grown into the rotifer whose shell will be discarded after the contents are consumed. Drawn from culture.

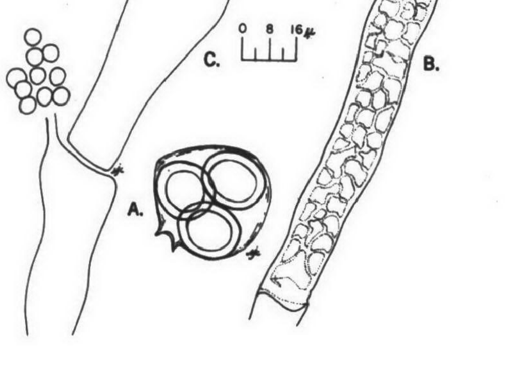
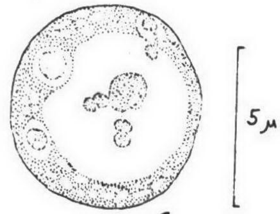


Figure 5

***Achlya americana* Humphrey**

Ooogonium with three oospores (A); young zoosporangium with delimited zoospores (B); and zoosporangia (C) with released zoospores that remain encysted in clusters at the mouth of the discharge tube. Drawn from culture.



6

Dermocystidium marinum

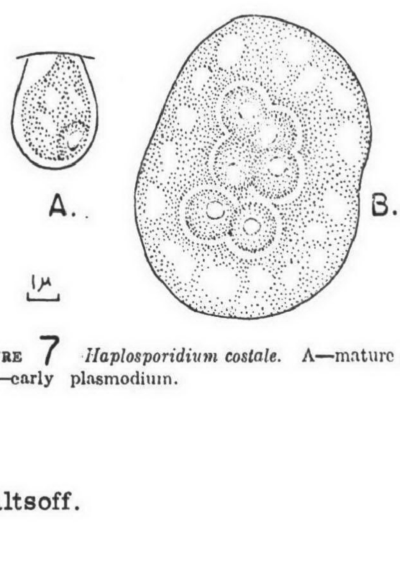


FIGURE 7 *Haplosporidium costale.* A—mature spore; B—early plasmodium.

Figures 1 through 5 from Cooke; Figures 6 and 7 from Galtsoff.

sugars and grows most luxuriantly in the presence of organic nitrogenous wastes.

3 Ecological roles

Although the "sewage fungi" on occasion attain visually noticeable concentrations, the less obvious populations of deuteromycetes may be more important in the ecology of the aquatic habitat. Investigations of the past decade indicate that numerous fungi are of primary importance in the mineralization of organic wastes, the overall significance and exact roles of fungi in this process are yet to be established.

D Predacious Fungi

1 Zoophagus insidians

(Plate II, Figure 4) has been observed to impair functioning of laboratory activated sludge units (see Cooke and Ludzack).

2 Arthrobotrys is usually found along with Zoophagus in laboratory activated sludge units. This fungus is predacious upon nematodes. Loops rather than "pegs" are used in snaring nematodes.

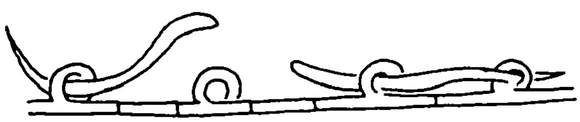


PLATE II (Figure 4)

IV CLASSIFICATION

In recent classification schemes, classes of fungi are distinguished primarily on the basis of the morphology of the sexual and zoosporic stages. In practical schematics, however, numerous fungi do not demonstrate these stages. Classification must therefore be based on the sum total of the morphological and/or physiological characteristics. The extensive review by Cooke (1963) on methods of isolation and classification of fungi from sewage and polluted waters precludes the need herein of extensive keys and species illustrations. A brief synopsis key of the fungi adapted in part from Alexopholous (1962) is presented on the following pages.

This outline was prepared by Dr. Donald G. Ahearn, Professor of Biology, Georgia State College, Atlanta, Georgia 30303.

KEY TO THE MAJOR TAXA OF FUNGI

1 Definite cell walls lacking, somatic phase a free living Plasmodium
Sub-phylum Myxomycotina (true slime molds) Class Myxomycetes

1' Cell walls usually well defined, somatic phase not a free-living Plasmodium
(true fungi) Sub-phylum Eumycotina 2

2 Hyphal filaments usually coenoctytic, rarely septate, sex cells when present forming oospores or zygospores, aquatic species propagating asexually by zoospores, terrestrial species by zoospores, sporangiospores conidia or conidia-like sporangia "Phycomycetes" 3

The phycomycetes are generally considered to include the most primitive of the true fungi As a whole they encompass a wide diversity of forms with some showing relationships to the flagellates, while others closely resemble colorless algae, and still others are true molds The vegetative body (thallus) may be non-specialized and entirely converted into a reproductive organ (holocarpic), or it may bear tapering rhizoids, or be mycelial and very extensive The outstanding characteristics of the thallus is a tendency to be nonseptate and, in most groups, multinucliate, cross walls are laid down in vigorously growing material only to delimit the reporductive organs The spore unit of nonsexual reproduction is borne in a sporangium, and, in aquatic and semiaquatic orders, is provided with a single posterior or anterior flagellum or two laterally attached ones Sexual activity in the phycomycetes characteristically results in the formation of resting spores

2' (1') Hyphal filaments when present septate, without zoospores, with or without sporangia, usually with conida, sexual reproduction absent or culminating in the formation of asci or basidia 8

3 (2) Flagellated cells characteristically produced 4

3' Flagellated cells lacking or rarely produced 7

4 (3) Motile cells uniflagellate 5

4' Motile cells biflagellate 6

5 (4) Zoospores posteriorly uniflagellate, formed inside the sporangium class Chytridiomycetes

The Chytridiomycetes produce asexual zoospores with a single posterior whiplash flagellum The thallus is highly variable, the most primitive forms are unicellular and holocarpic and in their early stages of development are plasmodial (lack cell walls), more advanced forms develop rhizoids and with further evolutionary progress develop mycelium The principle chemical component of the cell wall is chitin, but cellulose is also present Chytrids are typically aquatic organisms but may be found in other habitats Some species are chitinolytic and/or keratinolytic Chytrids may be isolated from nature by baiting (e g hemp seeds or pine pollen) Chytrids occur both in marine and fresh water habitats and are of some economic importance due to their parasitism of algae and animals The genus *Dermocystidium* may be provisionally grouped with the chytrids Species of this genus cause serious epidemics of oysters and marine and fresh water fish

5' Zoospores anteriorly uniflagellate, formed inside or outside the sporangium class Hyphochytridiomycetes

These fungi are aquatic (fresh water or marine) chytrid-like fungi whose motile cells possess a single anterior flagellum of the tinsel type (feather-like) They are parasitic on algae and fungi or may be saprobic Cell walls contain chitin with some species also demonstrating cellulose content Little information is available on the biology of this class and at present it is limited to less than 20 species

6 (4') Flagella nearly equal, one whiplash the other tinsel class Oomycetes

A number of representatives of the Oomycetes have been shown to have cellulosic cell walls The mycelium is coenocytic, branched and well developed in most cases The sexual process results in the formation of a resting spore of the oogamous type, i e , a type of fertilization in which two heterogametangia come in contact and fuse their contents through a pore or tube The thalli in this class range from unicellular to profusely branched filamentous types Most forms are eucarpic, zoospores are produced throughout the class except in the more highly advanced species Certain species are of economic importance due to their destruction of food crops (potatoes and grapes) while others cause serious diseases of fish (e g *Saprolegina parasitica*) Members of the family Saprolegniaceae are the common water molds and are among the most ubiquitous fungi in nature The order Lagenidiales includes only a few species which are parasitic on algae, small animals, and other aquatic life The somatic structures of this taxon are holocarpic and endobiotic The sewage fungi are classified in the order Leptomitales Fungi of this order are characterized by the formation of refractile constrictions 'cellulin plugs ' occur throughout the thalli or, at least, at the bases of hyphae or to cut off reproductive structures Leptomitus lacteus may produce rather extensive fouling flocs or slimes in organically enriched waters

6' Flagella of unequal size both whiplash class Plasmodiophoromycetes

Members of this class are obligate endoparasites of vascular plants, algae and fungi The thallus consists of a plasmodium which develops within the host cells Nuclear division at some stages of the life cycle is of a type found in no other fungi but known to occur in protozoa Zoosporangia which arise directly from the plasmodium bear zoospores with two unequal anterior falgella The cell walls of these fungi apparently lack cellulose

7 (3') Mainly saprobic sex cell when present a zygospore class Zygomycetes

This class has well developed mycelium with septa developed in portions of the older hyphae, actively growing hyphae are normally non-septate The asexual spores are non-motile sporangiospores (aplanospores) Such spores lack flagella and are usually aerialy disseminated Sexual reproduction is initiated by the fusion of two gametangia with resultant formation of a thick-walled, resting spore, the zygospore In the more advanced species the sporangia or the sporangiospores are conidia-like Many of the Zygomycetes are of economic importance due to their ability to synthesize commercially valuable organic acids and alcohols, to transform steroids such as cortisone and to parasitize and destroy food crops A few species are capable of causing disease in man and animals (zygomycosis)

7' Obligate commensals of arthropods, zygospores usually lacking class Trichomycetes

The Trichomycetes are an ill-studied group of fungi which appear to be obligate commensals of arthropods The trichomycetes are associated with a wide variety of insecta diplopods, and crustacea of terrestrial and aquatic (fresh and marine) habitats None of the members of this class have been cultured in vitro for continued periods of times with any success Asexual reproduction is by means of sporangiospores Zygospores have been observed in species of several orders

8 (2') Sexual spores borne in asci class Ascomycetes

In the Ascomycetes the products of meiosis, the ascospores, are borne in sac like structures termed asci The ascus usually contains eight ascospores, but the number produced may vary with the species or strain Most species produce extensive septate mycelium This large class is divided into two subclasses on the presence or absence of an ascocarp The Hemiascomycetidae lack an ascocarp and do not produce ascogenous hyphae, this subclass includes the true yeasts The Euascomycetidae usually are divided into three series (Plectomycetes, Pyrenomycetes, and Discomycetes) on the basis of ascocarp structure

8' Sexual spores borne on basidia class Basidiomycetes

The Basidiomycetes generally are considered the most highly evolved of the fungi Karyogamy and meiosis occur in the basidium which bears sexual exogenous spores, basidiospores The mushrooms toadstools, rusts, and smuts are included in this class

8'' Sexual stage lacking Form class (Fungi Imperfecti) Deuteromycetes

The Deuteromycetes is a form class for those fungi (with morphological affinities to the Ascomycetes or Basidiomycetes) which have not demonstrated a sexual stage The generally employed classification scheme for these fungi is based on the morphology and color of the asexual reproductive stages This scheme is briefly outlined below Newer concepts of the classification based on conidium development after the classical work of S J Hughes (1953) may eventually replace the gross morphology system (see Barron 1968)

KEY TO THE FORM-ORDERS OF THE FUNGI IMPERFECTI

1	Reproduction by means of conidia, oidia, or by budding	2
1'	No reproductive structures present	Mycelia Sterilia
2 (1)	Reproduction by means of conidia borne in pycnidia	Sphaeropsidales
2'	Conidia, when formed, not in cycnidia	3
3 (2')	Conidia borne in acervuli	Melanconiales
3'	Conidia borne otherwise, or reproduction by oidia or by budding	Moniliales

KEY TO THE FORM-FAMILIES OF THE MONILIALES

1	Reproduction mainly by unicellular budding, yeast-like, mycelial phase, if present, secondary, arthrospores occasionally produced, manifest melanin pigmentation lacking	2
1'	Thallus mainly filamentous, dark melanin pigments sometimes produced	3
2 (1)	Ballistospores produced	Sporobolomycetaceae
2'	No ballistospores	Cryptococcaceae
3	Conidiophores, if present, not united into sporodochia or synnemata	4
3'	Sporodochia present	Tuberculariaceae
3''	Synnemata present	Stilbellaceae
4 (3)	Conidia and conidiophores or oidia hyaline or brightly colored	Moniliaceae
4'	Conidia and/or conidiophores, containing dark melanin pigment	Dematiaceae

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FRESHWATER POLLUTION ECOLOGY

Q. WHAT IS ECOLOGY?

A. The science of the interrelation between living organisms and their environment.

Q. WHAT IS NOT ECOLOGY?

A. Not much!

T. T. Macan

SECTION E

WATER QUALITY AFFECTS AQUATIC COMMUNITIES

In this section a series of outlines identify typical changes in aquatic communities as a response to a variety of insults. Obvious changes may take place in population numbers of a single species as well as in community balance. Biology may be used for the characterization of water quality and interpretation of population trends within the biota. The final outline is a review of past and current efforts to relate water quality of biological communities.

Contents of Section E

BIOLOGICAL ASPECTS OF NATURAL SELF PURIFICATION

I INTRODUCTION

A *The results of* natural self purification processes are readily observed. Did they not exist, sewage (and other organic wastes) would forever remain, and the world as we know it would long ago have become uninhabitable. Physical, chemical, and biological factors are involved. The microscopic and macroscopic animals and plants in a body of water receiving organic wastes are not only exposed to all of the various (ecological) conditions in that water, but they themselves create and profoundly modify certain of those conditions.

B Since toxic chemicals kill some of or all of the aquatic organisms, their presence disrupts the natural self purification processes, and hence, will not be considered here. The following discussion is based solely on the effects of organic pollution such as sewage or other readily oxidizable organic wastes.

C This description is based on the concept of a "stream" since under the circumstances of stream or river flow, the events and conditions occur in a linear succession. The same fundamental processes occur in lakes, estuaries, and oceans, except that the sequence of events may become telescoped or confused due to the reduction or variability of water movements.

D The particular biota (plants and animals, or flora and fauna) employed as illustrations below are typical of central United States. Similar or equivalent forms occur in similar circumstances in other parts of the world.

E This presentation is based on an unpublished chart produced by Dr. C.M. Tarzwell and his co-workers in 1951. Examples from this chart are employed in the presentation.

II THE STARTING POINT

A A normal unpolluted stream is assumed as a starting point. (Figure 1)

B The cycle of life is in reasonably stable balance.

C A great variety of life is present, but no one species or type predominates.

D The organisms present are adjusted to the normal ranges of physical and chemical factors characteristic of the region, such as the following

1 The latitude, turbidity, typical cloud cover, etc. affect the amount of light penetration and hence photosynthesis.

2 The slope, cross sectional area, and nature of the bottom affect the rate of flow, and hence the type of organisms present deposition of sludge, etc.

3 The temperature affects both certain physical characteristics of the water, and the rate of biological activity (metabolism).

4 Dissolved substances naturally present in the water greatly affect living organisms (hard water vs. soft water fauna and flora).

E Clean water zones can usually be characterized as follows·

1 General features·

a Dissolved oxygen high

b BOD low

c Turbidity low

d Organic content low

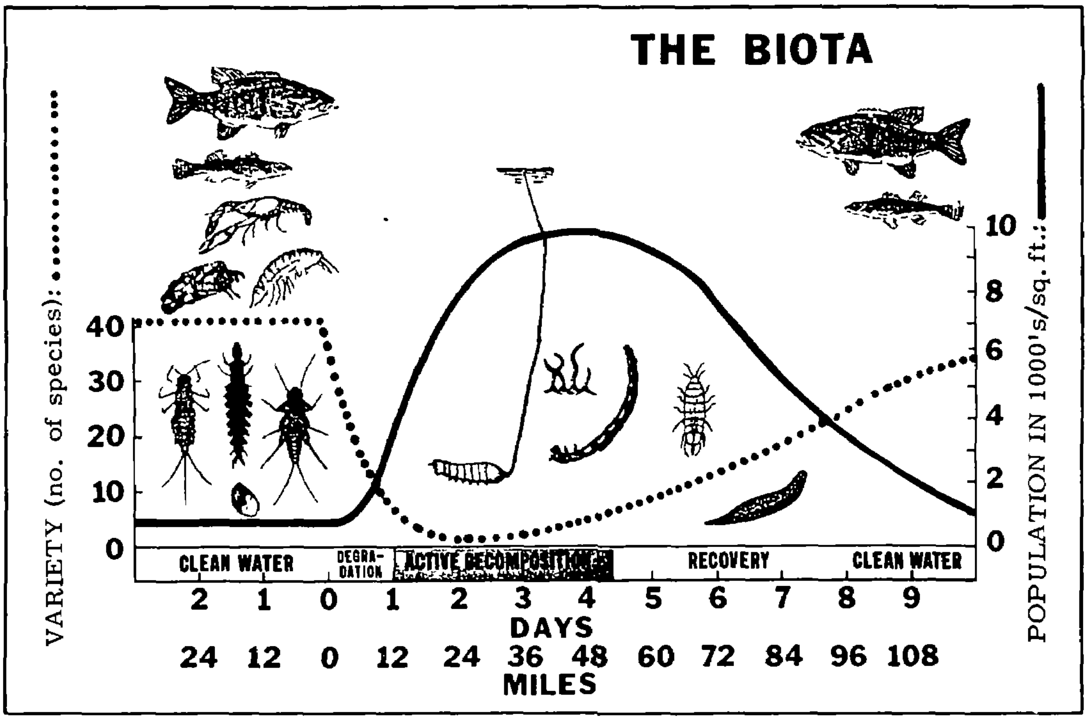

Figure 1· Relations between variety and abundance (production) of aquatic life, as organic pollution (discharged at mile 0) is carried down a stream. Time and distance scales are only relative and will be found to differ in nearly every case. After Bartsch and Ingram.[1]

e Bacterial count low

f Numbers of species high

g Numbers of organisms of each species moderate or low

h Bottom free of sludge deposits

2 Characteristic biota includes a wide variety of forms such as·

a A variety of algae and native higher (vascular, or rooted) plants

b Caddis fly larvae (Trichoptera)

c Mayfly larvae (Ephemeroptera)

d Stonefly larvae (Plecoptera)

e Damselfly larvae (Zygoptera)

f Beetles (Coleoptera)

g Clams (Pelecypoda)

h Fish such as

- Minnows (Notropid types)
- Darters (Etheostomatidae)
- Millers thumb (Cottidae)
- Sunfishes and basses (Centrarchidae)
- Sauger, yellow perch, etc. (Percidae)
- Others

3 Organisms characteristic of clean lakes, estuaries, or oceanic shores might be substituted for the above, and likewise in the following sections. However, it should be recognized that no single habitat is as thoroughly understood in this regard as the freshwater stream.

III POLLUTION

A With the introduction or organic pollution (Figure 1, day 0), a succession of fairly well organized events is initiated. Important items to observe in interpreting the pollutional significance of stream organisms are the following

B Numbers of species present, they tend to decrease with pollution.

C Numbers of individuals of each species tends to increase with pollution.

D Ratios between types of organisms are disturbed by pollution.

1 Clean water species intolerant of organic pollution tend to become scarce and unhealthy.

2 Animals with air breathing devices or habits tend to increase in numbers.

3 Scavengers become dominant

4 Predators disappear

5 Higher plants, green algae, and most diatoms tend to disappear.

6 Blue green algae often become conspicious

E The importance of observations on any single species is very slight.

IV THE ZONE OF RECENT POLLUTION

A The zone of recent pollution begins with the act of pollution, the introduction of excessive organic matter food for microorganisms (Figure 1, day 0)

B There follows a period of physical mixing.

C Many animals and plants are smothered or shaded out by the suspended material.

D With this enormous new supply of food material, bacteria and other saprophytic microorganisms begin to increase rapidly.

E The elimination of intolerant predatory animals allows the larger scavengers to take full advantage of the situation.

F This explosive growth of organisms, particularly fungi and bacteria, draws heavily on the free dissolved oxygen for respiration, and may eventually eliminate it.

G The number of types of organisms diminishes but numbers of individuals of tolerant types may increase.

H Zone of degeneration, or recent pollution, can usually be characterized as follows:

1 General features:

a DO variable, 2 ppm to saturation

b BOD high

c Turbidity high

d Organic content high

e Bacterial count variable to high

f Number of species declines from clean water zone

g Number of organisms per species tends to increase

h Other· Slime may appear on bottom

2 Characteristic biota

a Fewer higher plants, but rank heavy growth of those which persist

b Increase in tolerant green, and blue green algae

c Midge larvae (Chironomidae) may become extremely abundant

d Back swimmers (Corixidae) and water boatmen (Notonectidae) often present

e Sludge worms (Tubificidae) common to abundant.

f Dragonflies (Anisoptera) often present have unique tail breathing strainer

g Fish types, eg

- Fathead minnows (Pimephales promelas)

- White sucker (Catostomus commersonni)

- Bowfin (Amia calva)

- Carp (Cyprinus carpio)

V THE SEPTIC ZONE

A The exact location of the beginning of the septic zone, if one occurs, varies with season and other circumstances. (Figure 1, day 1)

B Lack of free DO kills many microorganisms and nearly all larger plants and animals, again replenishing the mass of dead organic material.

C Varieties of both macro and micro-organisms and adjustable types (facultative) that can live in the absence of free oxygen (anaerobic) take over.

D These organisms continue to feed on their bonanza of food (pollution) until it is depleted.

E The numbers of types of organisms is now at a minimum, numbers of individuals may or may not be at a maximum.

F The septic zone, or zone of putrefaction can usually be characterized as follows:

1 General features.

a Little or no DO during warm weather

b BOD high but decreasing

c Turbidity high, dark, odoriferous

d Organic content high but decreasing

e Bacterial count high

f Number of species very low

g Number of organisms may be extremely high

h Other. Slime blanket and sludge deposits usually present, oily appearance on surface, rising gas bubbles

2 Characteristic biota

a Blue green algae

b Mosquito larvae

c Rat-tailed maggots

d Sludge worms (Tubificidae and similar forms). Small, red, segmented (annelid) worms seem to be characteristic of this zone in both fresh and salt waters, the world around.

e Air breathing snails (Physa for example)

f Fish types: None

3 Note Fortunately, all polluted waters do not always degenerate to "septic" conditions.

VI THE RECOVERY ZONE

A The septic zone gradually merges into the recovery zone. (Figure 1, day 4)

B As the excessive food reserves diminish so do the numbers of anaerobic organisms and other pollution tolerant forms.

C As the excessive demand for oxygen diminishes, free DO begins to appear and likewise oxygen requiring (aerobic) organisms.

D As the suspended material is reduced and available mineral materials increase due to microbial action, algae begin to increase often in great abundance.

E Photosynthesis by the algae releases more oxygen, thus hastening recovery.

F Since algae require oxygen at all times for respiration (like animals), heavy concentrations of algae will deplete free DO during the night when it is not being replenished by photosynthesis.

G Consequently this zone is characterized by extreme diurnal fluctuations in DO.

H With oxygen for respiration and algae, etc. for food, general animal growth is resumed.

I The stream may now enter a period of excessive productivity which lasts until the accumulated energy (food) reserves have been dissipated.

J Zone of recovery may usually be characterized as follows:

1 General features

a DO 2 ppm to saturation

b BOD dropping

c Turbidity dropping, less color and odor

d Organic content dropping

e Bacterial count dropping

f Numbers of species increasing

g Numbers of organisms per species decreasing, (with the increase in competition)

h Other: Less slime and sludge

2 Characteristic biota

a Blue green algae

b Tolerant green flagellates and other algae

c Rooted higher plants in lower reaches

d Midge larve (Chironomids)

e Black fly larvae (Simulium)

f Giant water bugs (Belostoma spp.)

g Clams (Megalonais)

h Fish types

- Green sunfish (Lepomis cyanellus)

- Common sucker (Catostomus commersonni)

- Flathead catfish (Pylodictis olivaris)

- Stoneroller minnow (Campostoma anomalum)

- Buffalo (Ictiobus cyprinellus)

3 Excessive production and extreme variability often characterize middle and lower recovery zones.

4 Unfortunately, many waters once polluted never completely "recover". Re-pollution is the rule in many areas so that after the initial pollution, clear out delineation of zones is not possible. Characterization of these waters may involve such parameters as productivity, BOD, some "index" figure, or other value not included here.

VII CLEAN WATER ZONE

A Clean water conditions again obtain when productivity has returned to a normal, relatively poor level, and a well balanced varied flora and fauna are present. (Figure 1, day "10") Conditions may usually be characterized as follows·

B General features· similar to upstream clean water except that it is now a larger stream.

C Characteristic biota· similar to upstream clean water fauna and flora except that species include those indigenous to a larger stream.

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This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268.

ECOLOGY OF WASTE STABILIZATION PROCESSES

I INTRODUCTION

Living organisms will live where they can live. This holds for treatment plant environments just as it does for streams, impoundments, oceans, dry or wet lands.

A Each species has certain limits or tolerances, growth, feeding habits and other characteristics that determine its favored habitat.

B The presence of certain organisms with well defined characteristics in a viable condition and in significant numbers also provides some inference with respect to the habitat.

C The indicator organism concept has certain pitfalls. It is not sufficient to base an opinion upon one or more critters which may have been there as a result of gas liquid or solid transport. It is necessary to observe growth patterns, associated organisms, environmental conditions, and nutritional characteristics to provide information on environmental acceptability.

D Organisms characteristic of wastewater treatment commonly are those found in nature under low DO conditions. Performance characteristics are related to certain organism progressions and associations that are influenced by food to organism ratios and pertinent conditions. One single species is unlikely to perform all of the functions expected during waste treatment. Many associated organisms compete in an ecological system for a favored position. The combination includes synergistic, antagonistic, competitive, predative, and other relationships that may favor predominance of one group for a time and other groups under other conditions.

E It is the responsibility of the treatment plant control team to manage conditions of treatment to favor the best attainable performance during each hour of the day each day of the year. This outline considers certain biological characteristics and their implications with respect to treatment performance.

II TREATMENT PLANT ORGANISMS

Wastewater is characterized by overfertilization from the standpoint of nutritional elements, by varying amounts of items that may not enter the metabolic pattern but have some effect upon it, such as silt, and by materials that will interfere with metabolic patterns. Components vary in availability from those that are readily acceptable to those that persist for long periods of time Each item has some effect upon the organism response to the mixture.

A Slime forming organisms including certain bacteria, fungi, yeasts, protista monera and alga tend to grow rapidly on dissolved nutrients under favorable conditions. These grow rapidly enough to dominate the overall population during early stages of growth. There may be tremendous numbers of relatively few species until available nutrients have been converted to cell mass or other limiting factors check the population explosion.

B Abundant slime growth favors production of predator organisms such as amoeba or flagellates. These feed upon preformed cell mass. Amoebas tend to flow around particulate materials; flagellates also are relatively inefficient food gatherers. They tend to become numerous when the nutrient level is high. They are likely to be associated with floculated masses where food is more abundant.

C Ciliated organisms are more efficient food gatherers because they have the ability to move more readily and may set up currents in the water to bring food to them for ingestion. Stalked ciliates are implicated with well stabilized effluents because they are capable of sweeping the fine particulates from the water between floc masses while their residues tend to become associated with the floc.

D Larger organisms tend to become established later and serve as scavengers. These include Oligochaetes (worms), Chironomids (bloodworms and insect larvae), Isopods (sow bugs and crustacea), Rotifera and others.

III TREATMENT OPERATIONAL CONTROL

An established treatment plant is likely to contain representative organisms from all groups of tolerant species. Trickling filters, activated sludge, or ponds tend to retain previously developed organisms in large numbers relative to the incoming feed. The number and variety available determine the nature, degree and time required for partial oxidation and conversion of pollutants from liquid to solid concentrates.

A Proliferation of slime forming organisms characterize the new unit because they grow rapidly on soluble nutrients. Predators and scavengers may start growing as soon as cell mass particulates appear but growth rate is slower and numbers and mass lag as compared with slime organisms. As slime growth slows due to conversion of soluble nutrients to cell mass, the slime formers tend to associate as agglomerates or clumps promoting floculation and liquid solid separation.

B Overfeeding an established unit encourages rapid growth of slime organisms as individual cells rather than as flocculated masses. This results in certain characteristics resembling those of a young, rapidly growing system.

C Toxic feeds or unfavorable conditions materially reduce the population of exposed sensitive organisms. The net effect is a population selection requiring rapid regrowth to reestablish desired operating characteristics. The system assumes new growth characteristics to a degree depending upon the fraction remaining after the toxic effect has been relieved by dilution, degradation, sorption, or other means.

D Treatment units are characterized by changes in response to feed sequence, load ratio, and physical or chemical conditions. Response to accute toxicity may be immediately apparent. Chronic overloading or mild toxicity may not be apparent for several days. It may be expected that it will require 1 to 3 weeks to restore effective performance after any major upset. Performance criteria may not indicate a smooth progression toward improved operation.

E Observations of the growth characteristics and populations do not provide quantitative information, but they do indicate trends and stages of development that are useful to identify problems. It is not possible to identify most slime organisms by direct observation. It is possible to recognize growth and flocculation characteristics. Certain larger organisms are recognizable and are useful as indicator organisms to suggest past or subsequent developments.

IV ILLUSTRATIONS OF ECOLOGICAL SIGNIFICANCE

A The first group represents initial development of non-flocculent growth. Single celled and filamentous growth are shown. Rapid growth shows little evidence of flocculation that is necessary to produce a stable, clear effluent.

B The next group of slides indicate development of floc forming tendencies from filamentous or non-filamentous growth. Clarification and compaction characteristics are relatable to the nature and density of floc masses.

C Organisms likely to be associated with more stabilized sludges are shown in the third group. Scavengers essentially consist of a large alimentary canal with accessories.

D The last two slides illustrate changes in appearance after a toxic load. Scavengers, ciliates, etc. have been inactivated. New growth at the edge of the floc masses are not apparent. Physical structure indicates dispersed residue rather than agglomeration tendencies. The floc probably contains living organisms protected by the surrounding organic material, but only time and regrowth will reestablish a working floc with good stabilization and clarification tendencies.

ACKNOWLEDGEMENTS

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This outline was prepared by F J Ludzack, Chemist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

EFFECTS OF POLLUTION ON FISH

I INTRODUCTION

A By what means do pollutants exert their effects?

B What is the relationship between water quality and water use by fishes?

C What is the reaction of fishes to domestic sewage?

D Is there any noticeable change in species composition of the population following pollution?

E Is there any genetic or environmental selection in favor of pollution resistant strains?

F Nearly any pollutant, given sufficient concentration and time, can kill as a direct toxicant. We are primarily concerned here with sub-lethal or chronic levels of pollutants. (Acute toxic levels and physiological mechanisms are treated elsewhere.)

II MECHANISMS OF DETRIMENTAL ACTION

A Inert silt may

1 Clog gills and smother eggs and fry,

2 Blind sight feeders and eliminate hiding places,

3 Smother food organisms,

4 Reduce oxygenation by smothering algae.

B Irritants may

1 Act as repellents,

2 Cause excessive mucous secretion and upset osmotic balance.

C Sub-lethal quantities of a host of environmental materials are constantly penetrating the bodies of fishes by various routes. We are generally not aware of their presence unless they:

1 Cause an observable effect on the fish,

2 Cause an effect on man by imparting off-taste or odor to fish flesh,

3 Are sought for and detected, e.g., radioactive substances, DDT, mercury.

We can only speculate as to their undetected effects.

III ENVIRONMENTAL RELATIONSHIPS BETWEEN WATER QUALITY AND WATER USE BY FISHES

A Freshwater fishes sometimes spend their entire lives in a single body of water. Pollution of that body of water therefore impinges on them at every stage of their life cycle, and at every point in their various ecological relationships; such as, seeking food or escaping enemies.

B Migratory fishes on the other hand feed and grow up in one body of water (the ocean for anadromous species, fresh water for the catadronous eels), then travel a migration route (usually a river) to another body of water where they breed.

Pollution at either end of the route, or a pollution block along the migration route, may eliminate the species.

C What will affect one species adversely may be favorable for another.

1 Cold water species, such as various trouts, might be killed or eliminated by warmed water from a power plant which would in turn permit the survival of warm water species, such as certain basses, sunfishes, etc.

2 Benthic species (such as catfish, sculpins, or suckers, which live near the bottom) might be eliminated by a smothering

blanket of inert material which would not affect limnetic species inhabiting the open water areas (such as white and yellow bass, gizzard shad, or walleye). The limnetic species on the other hand might be inhibited by a dense turbidity which would hide their prey, suppress the growth of nutritious plankton, or clog their gills, this in turn being relatively harmless to the benthic group.

3 Likewise the shoreline hugging littoral forms (like pumpkinseed or bluegills) and profundal species (such as lake trout) might respond selectively to such factors as temperature or transparency.

IV RESPONSE OF FISHES TO SEWAGE AND SIMILAR WASTES

A These wastes in general are not toxic in themselves, but exert their effects on fishes directly.

B Oxygen Depletion

1 May lead to death at various stages in life history depending on circumstances.

2 May lower resistance to disease or increase sensitivity to intoxication.

3 May reduce ability to capture food or swim against current.

C May smother or kill normal food sources.

D May increase normal fish production through eutrophication.

E Usually changes normal population balance by driving out predatory types and encouraging scavengers.

F Reported to cause osteological and other pathological manifestations, such as the knothead condition of carps in the Illinois River.

V NATURAL SELECTION AND ACCLIMATIZATION TO POLLUTION

A Known biological mechanisms for selective breeding of pollution resistant strains operate in nature among fishes as among other organisms.

1 Studies of population genetics indicate that after some finite number of generations of population stress (e.g.; exposure to a given pollutant), permanent heritable resistance may be expected to develop.

2 If the environmental stress (or pollutant) is removed prior to the time that permanent resistance is developed in the population, reversion to the non-resistant condition may occur within a relatively few generations.

3 Habitats harboring populations under stress in this manner are often marked with the dead bodies of the unsuccessful individuals.

B Individual organisms on the other hand can over a period of time (less than one life cycle) develop a limited ability to tolerate different conditions, e.g.; pollutants:

1 With reference to all categories of pollutants both relatively facultative and obligate species are encountered (e.g.; euryhaline vs. stenohaline, eurythermal vs. stenothermal).

2 This temporary somatic acclimatization is not heritable.

C A given single-species collection or sample of living fishes may therefore represent one or more types of pollution resistance:

1 A sample of an original population which has been acclimated to a given stress <u>in toto</u>.

2 A sample of the surviving portion of an original population, which has been "selected" by the ability to endure the stress. The dead fish in a partial fish kill are that portion of the original population unable to endure the stress.

3 A sample of a sub-population of the original species in question which has *in toto* over a period of several generations developed a heritable stress resistance.

D Any given multi-species field collection will normally contain species illustrative of one or more of the conditions outlined above.

VI POPULATION COMPOSITION RESPONSES TO POLLUTION

A Sewage pollution generally results in a reduction in the predatory types and their replacement by scavengers. Regions of severe oxygen depletion may be devoid of fish, or inhabited only by rough fish such as gar or carp. The general concept of a reduction of variety coupled with an increase in abundance in certain regions is as valid for fishes as for other groups.

B Population responses to toxic pollution are unpredictable except that reduction in variety is again almost sure to result.

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THE INTERPRETATION OF BIOLOGICAL DATA WITH REFERENCE TO WATER QUALITY

I INTRODUCTION

Sanitary engineers like to have data presented to them in a readily assimilable form and some of them seem a little impatient with biologists who appear unable to provide definite quantitative criteria applicable to all kinds of water conditions. I think the feeling tends to be that this is the fault of biologists, and if they would only pull themselves out of the scientific stone-age all would be well. I will try to explain here why I believe that biological data can never be absolute nor interpretable without a certain amount of expertise. In this respect biologists resemble medical men who make their diagnoses against a complex background of detailed knowledge. Anyone can diagnose an open wound but it takes a doctor to identify an obscure disease, and although he can explain how he does it he cannot pass on his knowledge in that one explanation. Similarly, one does not need an expert to recognize gross organic pollution, but only a biologist can interpret more subtle biological conditions in a water body, and here again he can explain how he does it, but that does not make his hearer a biologist. Beck (1957) said something similar at a previous symposium in Cincinnati in 1956.

II THE COMPLEXITY OF BIOLOGICAL REACTIONS TO WATER CONDITIONS

A Complexity of the Aquatic Habitat

The aquatic habitat is complex and consists not only of water but of the substrata beneath it, which may be only indirectly influenced by the quality of the water. Moreover, in biological terms, water quality includes such features as rate of flow and temperature regime, which are not considered of direct importance by the chemist.

To many animals and plants, maximum summer temperature or maximum rate of flow is just as important as minimum oxygen tension. The result is that inland waters provide an enormous array of different combinations of conditions, each of which has its own community of plants and animals, and the variety of species involved is very great. Thus, for example, Germany has about 6000 species of aquatic animals (Illies 1961a) and probably at least as many species of plants. Yet Europe has a rather restricted fauna because of the Pleistocene ice age, in most other parts of the world the flora and fauna are even richer.

B Distribution of Species and Environmental Factors

We know something about the way in which species are distributed in the various habitats, especially in the relatively much studied continent of Europe, but we have, as yet, little idea as to what factors or combination of factors actually control the individual species.

1 Important ecological factors

Thus, it is possible to list the groups of organisms that occur in swift stony upland rivers (rhithron in the sense of Illies, 1961b) and to contrast them with those of the lower sluggish reaches (potamon). Similarly we know, more or less, the different floras and faunas we can expect in infertile (oligotrophic) and fertile (eutrophic) lakes. We are, however, much less informed as to just what ecological factors cause these differences. We know they include temperature and its yearly amplitude; oxygen, particularly at minimal levels; plant nutrients, such as nitrate, phosphate, silica, and bicarbonate; other ions in solution, including calcium, chloride, and possibly hydrogen; dissolved organic matter, which is necessary for some bacteria and fungi and probably for some algae, the nature of the substratum; and current.

2 Complexity of interacting factors

We also know these factors can interact in a complex manner and that their action on any particular organism can be indirect through other members of the biota.

a Induced periphyton growths

Heavy growths of encrusting algae induced by large amounts of plant nutrients, or of bacteria induced by ample supplies of organic matter, can eliminate or decimate populations of lithophile insects by simple mechanical interference. But the change does not stop there· the growths themselves provide habitats for the animals, such as Chironomidae and Naidid worms, which could not otherwise live on the stones.

b Oxygen levels and depositing substrates

If oxygen conditions over a muddy bottom reach levels just low enough to be intolerable to leeches, tubificid worms, which the leeches normally hold in check, are able to build up to enormous numbers especially as some of their competitors (e.g. Chironomus) are also eliminated.

c Oxygen levels and non muddy substrates

One then finds the typical outburst of sludge worms, so often cited as indicators of pollution. This does not happen if the same oxygen tension occurs over sand or rock, however, as these are not suitable substrata for the worms. Many such examples could be given, but they would only be ones we understand; there must be a far greater number about which we know nothing.

d One must conclude, therefore, that quite simple chemical changes can produce far-reaching biological effects, that we only understand a small proportion of them; and that they are not always the same.

3 Classic examples

This seems like a note of despair, however, if water quality deviates too far from normal, the effects are immediately apparent. Thus, poisonous substances eliminate many species and may leave no animals (Hynes 1960); excessive quantities of salt remove all leeches, amphipods, and most insects and leave a fauna consisting largely of Chironomidae, caddis worms, and oligochaetes (Albrecht 1954) and excessive amounts of dissolved organic matter give rise to carpets of sewage fungus, which never occur naturally. Here no great biological expertise is needed, and there is little difficulty in the communication of results. It is when effects are slighter and more subtle that biological findings become difficult to transmit intelligibly to other disciplines.

III THE PROBLEMS IN PRESENTATION OF BIOLOGICAL RESULTS

Because of these difficulties various attempts have been made to simplify the presentation of biological findings, but to my mind none of them is very successful because of the complexity of the subject. Early attempts at systematization developed almost independently on the two sides of the Atlantic, although they had some similarities.

A Early Studies in the United States (Richardson and the Illinois River)

In America, there was a simple division into zones of pollution, e.g. degradation, septic, and recovery, which were characterized in broad general terms. This simple, textbook approach is summarized by Whipple et al. (1947), and serves fairly well for categorizing gross organic pollution such as has been mentioned above. It was, however, soon found by Richardson (1929) during his classical studies on the Illinois River that typical "indicators" of foul conditions, such as Tubificidae and Chironomus, were not always present where they would be expected to occur. This was an early indication that it is not the water quality itself that provides suitable conditions for "pollution faunas," but other, usually associated, conditions - in this instance deposits of rich organic mud. Such conditions may, in fact, be present in places where water quality in no way resembles pollution, e.g., upstream of weirs in trout streams where autumn leaves accumulate and decay and cause the development of biota typical of organically polluted water. Samples must therefore be judged against a background of biological knowledge. Richardson was fully aware of this and was in no doubt about the condition of the Illinois River even in places where his samples showed few or no pollution indicators.

B The European Saprobic System

In Europe, the initial stress was primarily on microorganisms and results were first codified in the early years of the century by Kolkwitz and Marsson. In this "Saprobiensystem," zones of organic pollution similar to those described by the American workers were defined and organisms were listed as characteristic of one or more zones;

TABLE 1

SAPROBIENSYSTEM - A European system of classifying organisms according to their response to the organic pollution in slow moving streams. (22)

Alpha-Mesosaprobic Zone - Area of active decomposition, partly aerobic, partly anaerobic, in a stream heavily polluted with organic wastes.

Beta-Mesosaprobic Zone - That reach of stream that is moderately polluted with organic wastes.

Oligosaprobic Zone - That reach of a stream that is slightly polluted with organic wastes and contains the mineralized products of self-purification from organic pollution, but with none of the organic pollutants remaining.

Polysaprobic Zone - That area of a grossly polluted stream which contains the complex organic wastes that are decomposing primarily by anaerobic processes.

A recent exposition of this list is given by Kolkwitz (1950). It was then claimed that with a list of the species occurring at a particular point it was possible to allocate it to a saprobic zone. This system early met with criticism for several reasons. First,

TABLE 2

SAPROBICITY LEVELS ACCORDING TO THE TROPHIC STRUCTURE OF THE COMMUNITIES OF ORGANISMS

Saprobicity Level	Structure of the Communities of Organisms
I β-oligosaprobic	Balanced relationship between producers, consumers and destroyers, the communities of organisms are poor in individuals but there is a moderate variety of species, small biomass and low bioactivity.
II α-oligosaprobic	Balanced relationship between producers, consumers and destroyers, communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
III β-mesosaprobic	Substantially balanced relationship between producers, consumers and destroyers, a relative increase in the abundance of destroyers and, accordingly, of the consumers living off them, communities of organisms are rich in individuals and species with a large biomass and high bioactivity.
IV α-mesosaprobic	Producers decline as compared with an increase in consumers and destroyers, mixotrophic and amphitrophic forms predominate among the producers, communities of organisms rich in individuals but poor in species with a large biomass and extremely high bioactivity; still only few species of macro-organisms; mass development of bacteria and bacteria-eating ciliates.
V β-polysaprobic	Producers drastically decline, communities of organisms are extremely rich in individuals but poor in species with a large biomass and high bioactivity; macrofauna represented only by a few species of tubificids and chironomids; as in IV these are in great abundance; mass development of bacteria and bacteria-eating ciliates.
VI α-polysaprobic	Producers are absent; the total biomass is formed practically solely by anaerobic bacteria and fungi; macro-organisms are absent, flagellates outnumber ciliates amongst the protozoa.

Saprobicity - "Within the bioactivity of a body of water, Saprobicity is the sum total of all those metabolic processes which are the antithesis of primary production. It is therefore the sum total of all those processes which are accompanied by a loss of potential energy." Part I, Prague Convention.

all the organisms listed occurred in natural habitats--they were not evolved in polluted water--and there was much doubt as to the placing of many of the species in the lists. The system, however, did serve to codify ecological knowledge about a long list of species along an extended trophic scale. Its weaknesses appeared to be merely due to lack of knowledge; such a rigid system took far too little account of the complexity of the reaction of organisms to their habitats. For instance, many organisms can be found, albeit rarely, in a wide range of conditions and others may occur in restricted zones for reasons that have nothing to do with water quality. We often do not know if organisms confined to clean headwaters are kept there by high oxygen content, low summer temperatures, or inability to compete with other species under other conditions. In the swift waters of Switzerland the system broke down in that some organisms appeared in more polluted zones than their position in the lists would indicate. Presumably here the controlling factor was oxygen, which was relatively plentiful in turbulent cold water. In a recent series of experiments, Zimmerman (1962) has proven that current alone has a great influence on the biota, and identically polluted water flowing at different speeds produces biotic communities characteristic of different saprobic levels. He finds this surprising, but to me it seems an expected result, for the reasons given above.

C Recent Advances in the Saprobic System

1 Perhaps Zimmerman's surprise reflects the deeply rooted entrenchment of the Saprobiensystem in Central Europe. Despite its obvious shortcomings it has been revised and extended. Liebmann (1951) introduced the concept of considering number as well as occurrence and very rightly pointed out that the community of organisms is what matters rather than mere species lists. But he did not stress the importance of extrinsic factors, such as current, nor that the system can only apply to organic pollution and that different types of organic pollution differ in their effects, e.g., carbohydrate solutions from paper works produce different results from those of sewage, as they contain little nitrogen and very different suspended solids. Other workers (Sladecek 1961 and references therein) have subdivided the more polluted zones, which now, instead of being merely descriptive, are considered to represent definite ranges of oxygen content, BOD, sulfide, and even E. coli populations. Every water chemist knows that BOD and oxygen content are not directly related and to assume that either should be more than vaguely related to the complexities of biological reactions seems to me to indicate a fundamental lack of ecological understanding. I also think it is damaging to the hope of mutual understanding between the various disciplines concerned with water quality to give the impression that one can expect to find a close and rigid relationship between water quality measurements as assessed by different sets of parameters. Inevitably these relationships vary with local conditions, what applies in a sluggish river in summer will certainly not apply to a mountain stream or even to the same river in the winter. Correlation of data, even within one discipline, needs understanding, knowledge, and judgment.

2 Caspers and Schulz (1960) showed that the failure of the system to distinguish between waters that are naturally productive and those artifically enriched can lead to absurd results. They studied a canal in Hamburg, which because of its urban situation can only be regarded as grossly polluted. Yet it develops a rich plankton,

the composition of which, according to the system, shows it to be virtually clean.

D Numerical Application of the Saprobic System

Once the Saprobiensystem was accepted it was logical to attempt to reduce its findings to simple figures or graphs for presentation of results. Several such methods were developed, which are described by Tumpling (1960), who also gives the original references. In all these methods, the abundance of each species is recorded on some sort of logarithmic scale (e.g. 1 for present, 3 for frequent, 5 for common, etc.) The sums of these abundances in each saprobic level are plotted on graphs, the two most polluted zones showing as negative and others as positive. Or, the various saprobic levels are given numerical values [1 for oligosaprobic (clean), 2 for β-mesosaprobic, etc.] and the rating for each species is multiplied by its abundance number. The sum of all these products divided by the sum of all the frequencies gives a "saprobic index" for the locality. Clearly the higher this number, the worse the water quality in terms of organic pollution. In a similar way the so-called "relative Belastung" (relative load) is calculated by expressing the sums of all the abundances of organisms characteristic of the two most-polluted zones as a percentage of the sum of all abundances. Then 100 percent is completely polluted water, and clean localities will give a low number.

E Weaknesses of the Saprobic System

There are various elaborations of these methods, such as sharing of species between zones and taking account of changes in base-line as one passes downstream. None of them, however, eliminates the basic weaknesses of the system nor the fact that, as Caspers and Schulz (1960) point out, there is little agreement between the various authors in the assignment of species to the different levels. Therefore, one gains a number or a figure that looks precise and is easily understood, but it is based on very dubious foundations.

F Comparative North American Systems

Similar systems are indigenous to North America, but were independently evolved.

1 Wurtz (1955) and Wurtz and Dolan (1960) describe a system whereby animals are divided into sensitive-to-pollution and non-sensitive (others are ignored), and also into burrowing, sessile, and foraging species (six classes).

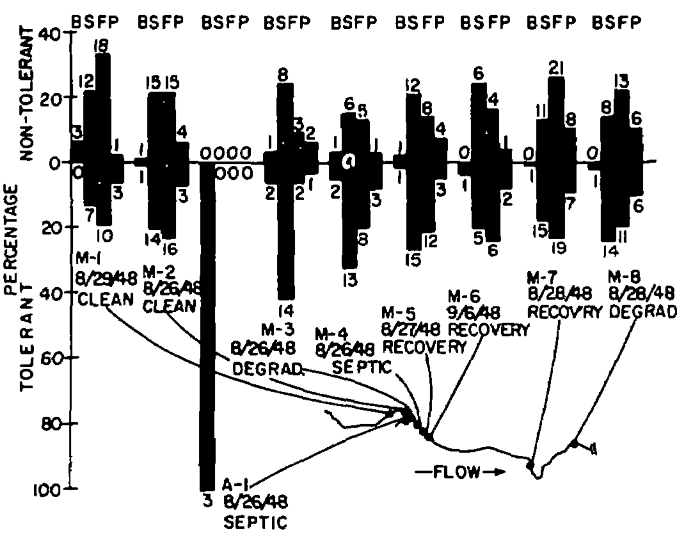

Figure 1. Histograms, based on selected organisms, illustrating stream reaches of clean, degradation, septic, and recovery conditions [after Wurtz] (22)

Numbers of these species represented are plotted for each station as six histograms on the basis of percentage of total number of species. If the constitution of the fauna from control stations or from similar localities is known, it is possible to express numerically "biological depression" (i.e., percentage reduction in total number of species), "biological distortion" (changes in proportions of tolerant and non-tolerant species), and "biological skewness" (changes in the ratios of the three habitat classes). Such results must, of course, be evaluated, and the definition of tolerance is quite subjective; but the method has the advantages of simplicity and dependence on control data. Like the Saprobiensystem, however, it can have no universal validity. It also suffers from the fact that it takes no account of numbers, a single specimen, which may be there by accident, carries as much weight as a dense population.

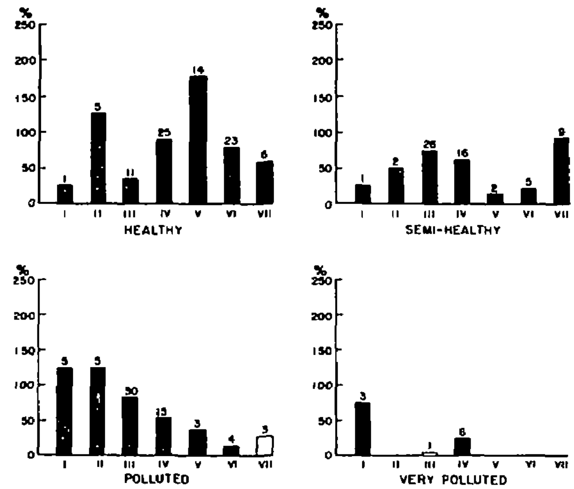

Figure 2. Histograms, based on selected organisms, illustrating healthy, semi-healthy, polluted, and very polluted stations in Conestoga Basin, Pa [after Patrick] (22)

2 Patrick (1949) developed a similar system in which several clean stations on the water body being investigated are chosen, and the average number of species is determined occurring in each of seven groups of taxa chosen because of their supposed reaction to pollution. These are then plotted as seven columns of equal height, and data from other stations are plotted on the same scale, it is assumed that stations differing markedly from the controls will show biological imbalance in that the columns will be of very unequal heights. Number is indicated by double width in any column containing species with an unusual number of individuals. I have already questioned the usefulness of this method of presentation (Hynes 1960), and doubt whether it gives any more readily assimilable data than simple tabulation, it does however, introduce the concept of ecological imbalance.

TABLE 3 —*Classification of Groups of Organisms Shown in Figure 2*

GROUP	ORGANISM
I	Blue-green algae, green algae of the genera *Stigeoclonium, Spirogyra*, and *Tribonema*, the bdelloid rotifers plus *Cephalodella megalocephala* and *Proales decipiens*
II	Oligochaetes, leeches, and pulmonate snails
III	Protozoa
IV	Diatoms, red algae, and "most of the green algae"
V	All rotifers not included in Group I, clams, gill breathing snails, and tricladid flatworms
VI	All insects and crustacea
VII	All fish

3 Beak (1964), another author, recognized the need for a concise expression of pollution based on biological information. Toward this end, he developed a method of biological scoring which is based on the frequency of occurrence of certain macroscopic invertebrates obtained from 6 years of study on one river. It will be noted that the Biological Score is a modification and expansion of Beck's Biotic Index.

The indicator organisms are divided into three categories: Group I contains the pollution-tolerant species, Group II comprises those species which are facultative with respect to pollution, and Group III contains the pollution-intolerant forms. Each group is assigned a weighted score that can be allotted to field samples on the following basis·

a Normal complement of Group III scores 3 points.

b Normal complement of Group II scores 2 points.

c Normal complement of Group I scores 1 point.

The scores are additive, thus an unpolluted stream will have a Biological Score of 6. If only pollution-tolerant forms are found, the score will be 1. If no organisms are found, the score will be zero. Furthermore, a score of, 1 or 2 points could be allotted to Group III when less than the normal complement is present Group II could be treated in a similar manner This scoring device correlated well with the biological oxygen demand, dissolved oxygen, and solids content of the receiving water Beak also related his scoring device to the fisheries potential. This relationship is shown in Table 4]

TABLE 4

TENTATIVE RELATIONSHIP OF THE BIOLOGICAL SCORE TO THE FISHERIES POTENTIAL (after Beak, 1964) (30)

Pollution status	Biotic index	Fisheries potential
Unpolluted	6	All normal fisheries for type of water well developed
Slight to moderate pollution	5 or 4	Most sensitive fish species reduced in numbers or missing
Moderate pollution	3	Only coarse fisheries maintained
Moderate to heavy pollution	2	If fish present, only those with high toleration of pollution
Heavy pollution	1	Very little, if any, fishery
Severe pollution, usually toxic	0	No fish

4 It has long been known that ecologically severe habitats contain fewer species than normal habitats and that the few species that can survive the severe conditions are often very abundant as they lack competitors Examples of this are the countless millions of Artemia and Ephydra in saline lakes and the Tubifex tubifex in foul mud This idea has often been expressed in terms of diversity, which is some measure of numbers of species divided by number of specimens collected Clearly, such a parameter is larger the greater the diversity, and hence the normality of the habitat Unfortunately, though as the number of species in any habitat is fixed, it also decreases as sample size increases so no index of diversity has any absolute value (Hairston 1959) If a definite sample size is fixed, however, in respect to numbers of organisms identified, it is possible to arrive at a constant index

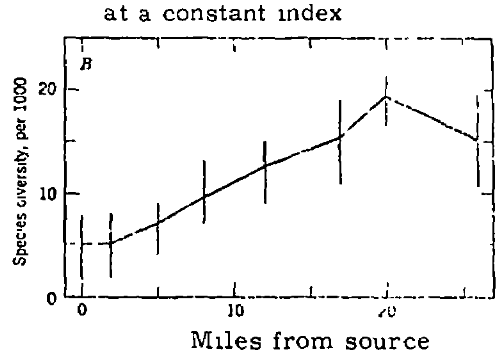

Figure 3 Zooplankton species diversity per thousand individuals encountered in marine systems affected by waste waters from petrochemical industrial wastes The vertical lines indicate seasonal variations. (30)

5 Patrick et al. (1954) in effect used this concept in a study of diatom species growing on slides suspended in water for fixed periods They identified 8000 specimens per sample and plotted the results as number of species per interval against number of specimens per species on a logarithmic scale This method of plotting gives a truncated normal curve for a wide variety of biotic communities In an ordinarily diverse habitat the mode is high and the curve short, i e , many species occur in small numbers and none is very abundant In a severe habitat the mode is low and the curve long, i e , there are few rare species and a few with large numbers This, again, seems to me to be an elaborate way of presenting data and to involve a lot of unnecessary arithmetic

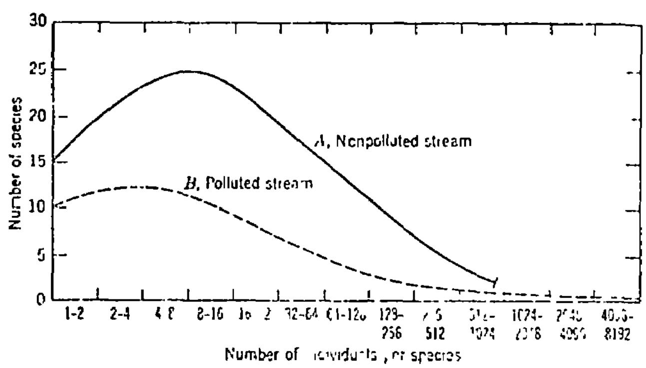

Figure 4. A graphic comparison of diatom communities from two different environments. (After Patrick et al , 1954) (30)

6 Diversity indices vs tabulated data

Allanson (1961) has applied this method to the invertebrate faunas of streams in South Africa and has shown, as has Patrick for diatoms, that the log normal curve is flatter and longer for polluted stations, the difference, however, is not so apparent that it does not need exposition Here, again, I would suggest that tabulated data are just as informative Indeed I would go further and say that tabulated data are essential in the present state of our knowledge We are learning as we go along and if the details of the basic findings are concealed by some sort of arithmetical manipulation they cannot be re-interpreted in the light of later knowledge, nor are they preserved in the store of human knowledge This point becomes particularly clear when one examines some of the early studies that include tables Butcher (1946) requotes a considerable amount of data he collected from studies of various English rivers during the thirties, they are not only clear and easy to follow, but they are also informative about the generalities of pollution in a way that data quoted only within the confines of some particular system are not

7 Examples of tabulated data(Table 5)

Simple tabulation of biological data in relation to water quality, either in terms of number of organisms, percentage composition of the biota, some arbitrary abundance scale, or as histograms, has been effectively practiced in many parts of the world in America (Gaufin and Tarzwell 1952, Gaufin 1958), Africa (Harrison 1958 and 1960, Hynes and Williams 1962), Europe (Albrecht 1954, Kaiser 1951, Hynes 1961, Hynes and Roberts 1962), and New Zealand (Hirsch 1958) to cite a few These tabulated data are easy to follow, are informative to the expert reader, and conceal no facts. Although the non-biologist may find them tedious, he need only read the explanatory paragraphs It is a delusion to think that it is possible to reduce biological data to simple numerical levels. At best, these can only be produced for limited situations and

TABLE 5

ORGANISMS	1			2			3		
	206.3 power line			205.3			203.3		
	left	mid	right	left	mid	right	left	mid	right
Zoothamnium	F								
Dendrosoma	C								F
Spongilla fragilis								A	
Trochospongilla leidyi	C			C					
Unidentified Sponge					F			A	
Cordylophora lacustris		F		F	A	A			
Dugesia tigrina	5		2	4	4	6	14		19
Urnatella gracilis	C	C	C	C	F		F		F
Paludicella articulata	C			F	F	F	F		F
Fredericella sultana	C								
Pristina							1		
Nais communis				1		1		7	
Paranais									
Unidentified Leech									1
Unidentified Beetle			1						
Chaoborus punctipennis					1				
Hydrobaenus sp A	31	47	5	9	117	68	1	26	4
Cricotopus bicinctus						1			
Unidentified Tendipedid								1	
Harnischia sp A		5	2		29	2	1	8	
Tendipes nervosus	1				4	15		2	1
Tendipes modestus	59	14	16	7	12	7	1		3
Polypedilum sp B									1
Calopsectra exigua			2	5	3	48	3	169	101
Trycorythodes	1			1			1		1
Stenonema				1					
Agraylea			2				1		
Athripsodes				2					
Potamyia flava				41		44	19	131	234
Hydropsyche orris				141		17	2	3	11
Cheumatopsyche		1		4	5	43		30	13
Psychomyiidae Genus A	51	22	21	30	54	92	11	193	21
Lithasia verrucosa									1
Ferrissia shimekii				4		33	7	5	19
Quadrula sp						1	1		
Quadrula tuberculata						2			
Corbicula fluminea	4	1	5	11		1	77		7
TOTAL	152	90	56	261	229	381	141	575	437
		298			871			1,153	

F - few C - common A - abundant

Benthos from Pickwick Tailwater (35)

even then they need verbal exposition, at worst, they give a spurious impression of having absolute validity

8 Comparison of stations

My final point in this section concerns comparisons It is claimed that the German system, in effect, measures an absolute state, a definite level of water quality We have seen that this is not a tenable claim In the other systems, by and large, the need to establish local control stations at which to measure the normal or "natural" biotic conditions is accepted, and then other areas are compared with this supposed norm. This is, of course not always possible as there may remain no unaffected area, or no unaffected area that is, with respect to such factors as current, nature of substratum, etc , sufficiently similar to act as a base-line for data Nevertheless, basically, these systems can be used to compare stations and thus to assess changes in water quality In doing this, they can all be used more or less successfully, but I maintain that a table is just as useful as an elaborate analysis, and I believe that the table should be included with whatever is done For a particular situation, however, it is often possible to distill the data into a single figure as a measure of similarity between stations

9 Coefficients of similarity

Burlington (1962) and Dean and Burlington (1963) have recently proposed an entirely objective means of doing this, which involves simple arithmetical manipulation In his system, a "prominence value" is calculated for each species at each station This is a product of its density and some function of its frequency in samples, but the details of this calculation can be altered to suit any particular situation Then a coefficient of similarity between each pair of stations can be calculated by dividing twice the sum of the lower prominence values of taxa that the two stations have in common by the sum of all the prominence values of both stations. Identical stations will then have a coefficient of similarity of 1·00, this coefficient will be lower the more the stations differ from one another. This is an easy way to compare stations in an entirely unbiased way and as such may satisfy the need for numerical exposition, however, it tells one nothing about why the localities are different and like all the other more or less numerical methods of presenting data has no absolute value Moreover, it still leaves unanswered the fundamental question of how different is "different?"

TABLE 6

Clean ↑ … Polluted ↓ — WATER QUALITY (high multiple use indicated) — Organisms in Order of Tendency to Disappear as Degree of Pollution Increases

Types of Organisms Present	BIOTIC INDEX[1] Variety Present	√Total Number of Groups Present 0-1	2-5	6-10	11-15	16+
			Bio	Index		
Plecoptera nymph present	More than one species	--	7	8	9	10
	One species only	--	6	7	8	9
Ephemeroptera nymph present	More than one species *	--	6	7	8	9 ✠
	One species only *	--	5	6	7	8
Trichoptera larvae present	More than one species •	--	5	6	7	8
	One species only •	4	4	5	6	7
Gammaridae present	All above species absent	3	4	5	6	7
Asellus and/or _Lirceus_ present	All above species absent	2	3	4	5	6
Tubificid worms, _Tendipes_, and _Cricotopus bicinctus_ (one or more of these groups)	All above species absent	1	2	3	4	--
All above types absent	Some organisms such as _Eristalis tenax_ not requiring dissolved oxygen may be present	0	1	2	--	--

* _Stenonema nepotellum_ excluded

10 ✠ main stream reservoirs and west Tennessee streams

• _Stenonema nepotellum_ (Ephem.) is counted in this section for the purpose of classification.

√ ONE FOR EACH KNOWN SPECIES IN THESE GROUPS:

- Platyhelminthes
- Hirudinea
- Mollusca
- Crustacea
- Plecoptera
- Diptera (excluding specific ones listed below)
- Coleoptera
- Neuroptera

√ ONE FOR EACH GROUP, REGARDLESS OF NUMBER OF SPECIES, ETC.:

- Annelida excluding Naididae
- Naididae
- Each Mayfly genera (excluding _Stenonema nepotellum_)
- _Stenonema nepotellum_
- Each Trichoptera family
- Chironomidae (excluding specific ones listed below)
- _Chironomus riparius_ and _plumosus_ and _Cricotopus bicinctus_.
- Family Simuliidae

[1] adapted from Trent River Board - Tennessee Stream Pollution Control Board 8/66 RMS

IV THE PROBLEMS OF SAMPLING

The systems outlined above are all based on the assumption that it is possible to sample an aquatic habitat with some degree of accuracy, this is a dubious assumption, however, when applied to biological data. From what has been said about the complexity of biological reactions to the various factors in the environment, and from the obvious fact that rivers especially are a mosaic of microhabitats, it is clear that to achieve numerical accuracy or even some limits of confidence considerable numbers of samples need to be taken. Indeed, even in so apparently unvaried a habitat as a single riffle, Needham and Usinger (1956) showed that a very large number of samples would be necessary to give significant numerical data.

A Representative Sampling

There is a limit to the number of samples that can reasonably be taken and, anyway, it is desirable to sample many different types of habitat so as to get as broad as possible an estimate of the biota This is the more recent approach of most of the workers in Central Europe, who have been content to cite abundances on a simple relative but arbitrary scale and to convert this to figures on some sort of logarithmic scale for use in calculations. An alternative is to express the catch in terms of percentage composition, but this has the disadvantage that micro- and macro-organisms cannot be expressed on the same scale as they are obtained by different collecting techniques. Also, of course, implicit in this approach is the assumption that the sampling is reasonably representative. Here again we run into the need for knowledge and expertise. In collection as well as in interpretation, the expert is essential. Biological sampling, unlike the simple, or fairly simple, filling of bottles for chemical analysis or the monitoring of measuring equipment, is a highly skilled job and not one to be handed over to a couple of vacationing undergraduates who are sent out with a Surber sampler and told to get on with it. This point has also been made by other biologists, e.g., Patrick (1961) who stresses the need for skilled and thorough collecting even for the determination of a species list.

B Non-Taxonomic Techniques

Alternatively we can use the less expert man when concentrating on only part of the habitat, using, say, microscopical slides suspended in the water to study algal growth. This method was extensively used by Butcher (1946), and Patrick et al.(1954) who studied diatoms in this way. This gives only a partial biological picture, but is useful as a means of monitoring a stretch of water where it is possible that changes might occur. It is a useful short-hand method, and as such is perhaps comparable to studying the oxygen absorbed from potassium permanganate instead of carrying out all the usual chemical analyses on water. A short method of this kind may serve very well most of the time, but, for instance, would not be likely to detect an insecticide in concentrations that could entirely eliminate arthropods and hence fishes by starvation.

C Monitoring

It is possible to work out biological monitoring systems for any specific purpose. The simplest of these is the cage of fish, which, like a single type of chemical analysis, can be expected to monitor only one thing -- the ability of fish to live in the water -- with no information on whether they can breed or whether there is anything for them to eat. Beak et al. (1959) describe a neat way in which the common constituents of the bottom fauna of Lake Ontario can be used to monitor the effluents from an industrial site. Obviously there is much room for such ingenuity in devising biological systems for particular conditions, but this is perhaps outside the scope of this meeting

V CONCLUSIONS

It may appear from the previous sections that my attitude to this problem is entirely obstructionist. This is far from being so. Water quality is as much biological phenomenon as it is a chemical or physical one, often what we want to know about water is almost exclusively biological -- will it smell nasty, is it fit to drink, can one bathe in it, etc? I suggest, therefore, that it is desirable to organize water monitoring programs that will tell one what one wants to know. There is no point in measuring everything biological, just as there is no point in performing every possible chemical analysis, what is measured should be related to local conditions. It would be a waste of time to measure oxygen content in a clean mountain stream, we know it to be high, and it becomes worth measuring only if we suspect that it may have been lowered by pollution. Similarly, there is little point in studying the plankton in such a stream, we know it only reflects the benthic flora. In a lake or in a slow river, on the other hand, if our interest in the water lies in its potability, records of the plankton are of considerable importance as changes in plankton are, in fact, changes in the usability of the water.

A Periphyton and Benthos Studies

For long-term studies, especially for the recording of trends or changes induced by pollution, altered drainage, agricultural poisons, and other havoc wrought by man, one can expect informative results from two principal techniques. First, we can study microscopic plant and animal growth with glass slides placed in the water for fixed periods, second, we can obtain random samples of the benthic fauna. The algae and associated microfauna tell one a good deal about the nutrient condition of the water and the changes that occur in it, and the larger benthic fauna reveal changes in the trophic status, siltation due to soil erosion, effects of insecticides and other poisons, etc.

B Varying Levels of Complexity

The study of growths on glass slides is reasonably skilled work, but can easily be taught to technicians, like chemical monitoring, such study needs to be done fairly often. Sampling the benthos is more difficult and, as explained above, needs expert handling, unlike most other monitoring programs, however, it need be done only infrequently, say, once or twice a year. Inevitably sampling methods will vary with type of habitat, in each case, the question will arise as to whether it is worth looking at the fish also. It is here that the biologist must exercise judgment in devising and carrying out the sampling program.

C Data Interpretation

Judgment is also needed in the interpretation of the data. It is for this reason I maintain that it should all be tabulated so that it remains available for reassessment or comparison with later surveys. If need be, some sort of numerical format can be prepared from the data for ad hoc uses, but it should never become a substitute for tabulations. Only in this way can we go on building up our knowledge. Perhaps some day we shall be able to pass all this information into a computer, which will then be able to exercise better judgment than the biologist. I hope this will happen, as computers are better able to remember and to cope with complexity than men. It will not, however, pension off the biologist. He will still be needed to collect and identify the samples. I cannot imagine any computer wading about on rocky riffles nor persuading outboard motors and mechanical grabs to operate from the unstable confines of small boats. We shall still need flesh and blood biologists long after the advent of the hardware water chemist, even though, with reference to my earlier analogy, a Tokyo University computer recently outpointed 10 veteran medicals in diagnosing brain tumors and heart disease. It should be pointed out, however, that the computer still had to be fed with information, so we are still a long way from the hardware general practitioner. I believe though that he is likely to evolve before the hardware biologist; after all, he studies only one animal.

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SECTION F

WATER QUALITY AND AQUATIC LIFE

Multiple usage of water is associated with a variety of concepts of acceptable quality. Water of quality suitable for one kind of use is not necessarily acceptable for another kind of use.

Included in the following outlines are sections from Water Quality Criteria (FWPCA 1968), and Water Research Needs (FWPCA 1969).

Contents of Section F

fish, other aquatic life, and wildlife

(Extracted from: Report of the Committee on
WATER QUALITY CRITERIA
FWPCA, USDI, April 1, 1968)

letter from the chairman

THE MEMBERSHIP of the National Technical Advisory Subcommittee on Water Quality Criteria for Fish, Other Aquatic Life, and Wildlife represents training and experience in several phases of freshwater, marine and wildlife ecology, physiology, and toxicology. The task of this Subcommittee is to describe, insofar as possible, under present knowledge: (1) the environmental requirements of aquatic life and wildlife, (2) the environmental concentrations of potential toxicants that are not harmful under long-term exposure, and (3) to suggest indirect methods for determining safe concentrations through bioassays and application factors. Because present knowledge of environmental requirements is incomplete and information on safe concentrations of toxicants is nonexistent for most organisms, the recommendations for water quality criteria of necessity are incomplete, tentative, and subject to change as additional information becomes available. In the determination of these criteria, the Subcommittee has utilized the broad knowledge, the many years of experience, and the understanding and commonsense of the Subcommittee members.

In order to expedite this task, the Subcommittee was divided into three groups: one for freshwater organisms; one for marine and estuarine organisms; and a third for wildlife.

Six task forces were set up in each of the first two groups. Each of these task forces was assigned certain environmental factors to review present knowledge and determine environmental conditions essential for the survival, growth, reproduction, general well-being, and production of a desired crop of aquatic organisms. Members assigned to each task force were experts on that particular subject or had wide experience with the factors or materials in question. They were selected with this in mind so that the whole subject could be covered most effectively. The composite report thus prepared was reviewed by the full Subcommittee and approved on October 31 and November 1, 1967, in Washington, D.C.

CLARENCE M. TARZWELL,
Chairman.

introduction

DURING the course of geologic time, organisms which were able to adapt so they were better fitted to live under existing environmental conditions were the ones which survived and now form the biota. Geologic change is a slow process and biota developed which were adapted not only to the physical and chemical but also to the biological factors of the environment. The environmental factors to which organisms adapted through the evolutionary process are now their environmental requirements. Therefore, any relatively rapid change in these conditions can be detrimental or even disastrous. Because the biota is the result of long evolutionary processes during which delicate balances were established, a change in conditions or in a portion of the biota can have far reaching effects.

Man has now attained the ability to alter drastically his environment and that of other organisms Many of his activities already have impaired seriously his own environment and that of other living things. Water pollution engineering works and other changes that modify the aquatic environment rank high in causing detrimental effects.

Water pollutants may be harmful through alterations in natural environmental conditions (such as temperature, dissolved oxygen, pH, carbonates, etc.), through physiological and other changes due to the addition of toxicants, or through both. Thus, in determining the effects of pollutants we must consider environmental, physiological, and accumulative effects.

Substances in suspension and solution, whether solid, liquid, or gas, largely determine the quality of the water. Aquatic organisms are affected not only directly by these materials, but also indirectly through their effects on other forms of aquatic life which comprise their food, competitors, and predators. Hence, the determination of water quality requirements for aquatic life is a very involved task. The problem is further complicated by the fact that different species and different developmental or life stages of the same or different species may differ widely in their sensitivity or tolerance to different materials, to ranges in environmental conditions, and to the cumulative synergistic and antagonistic effects of toxicants

In determining water quality requirements for aquatic life and wildlife, it is essential to recognize that there are not only acute and chronic toxic levels but also tolerable, favorable, and essential levels of dissolved materials. Lethal, tolerable, and favorable levels and conditions may be ascertained by: (1) determining the environmental factors and concentrations of materials which are favorable in natural waters; (2) determining by laboratory studies the relative sensitivity of organisms to various environmental factors, and ranges which are tolerable and favorable; (3) determining by means of different bioassay studies the behavioral, physiological, and other responses of organisms to potential toxicants and concentrations of these materials which are not harmful under continuous exposure; and (4) testing laboratory findings in the field to determine their adequacy for the protection of aquatic and wildlife resources.

In approaching this problem of protecting our aquatic and wildlife resources, it must also be realized that: (1) certain natural complexes of dissolved materials to which aquatic organisms have become adapted are favorable whereas other concentrations or compositions may not be; (2) unnatural materials added by man can be unfavorable; (3) altering the amounts of substances normally found in the environment can be harmful; (4) toxicity is a quantitative term—any material becomes toxic when its concentration exceeds certain levels. It is essential, also, to realize that requirements must be maintained throughout periods of low water, maximum discharge, maximum temperature, minimum DO, variations in pH, turbidity, salinity, etc Further, it should be understood that: (1) unfavorable conditions which may be resisted for long periods by adults may be entirely unfavorable for the survival of the species; (2) conditions need to be unfavorable for only a few hours to eliminate a population or group of species; and (3) levels of environmental factors and concentrations of toxicants that appear to cause no harm during a few hours of exposure may be intolerable for extended periods or for recurring short-term exposures.

In defining water quality requirements for aquatic life and wildlife, it is necessary to define the extreme upper and lower limits of the various environmental factors as well as the optimum values. These extremes are outer limits and constitute the minimum objectives to be obtained in the improvement of waters for aquatic life. It is not the intention of the Subcommittee that such levels are to be considered as satisfactory. Further, it is stressed that waters of higher quality should not be degraded towards approximation of the extremes. For example, the dissolved oxygen content of water should be near saturation for best production. The lower limits for oxygen indicated in the report, therefore, represents the objective to be obtained in the improvement of water, and not the level to which good waters may be lowered.

It is essential that the various recommendations be considered in context with the body of the report, taking due consideration of the variability of local conditions and native biota.

Within the United States there are great variations in environmental conditions and in the flora and fauna. The environmental requirements of the biota are different not only for different regions but for different portions of the same region Overlying these differences are seasonal changes and daily variations that have become essential factors in the environment. Ideally, therefore, water quality criteria for aquatic life and wildlife should take into consideration local variations in requirements, seasonal changes, and daily variations They should be national in scope They should be applicable to streams of various size and character, to all types of lakes, to reservoirs, estuaries, and coastal waters.

It is obvious that more research is needed on the character, conditions, and interrelations in fresh water, marine, and estuarine ecosystems which are subjected to degradation or alteration as well as on the physiological requirements and tolerances of the various species involved in these different ecosystems This need must be satisfied for the establishing of sound criteria to maintain and preserve aquatic resources and to permit the most economical and productive use of these resources by man.

Further, water quality requirements must be expressed so as to allow for environmental modifications where such modifications are justifiable and deemed to be in the public interest

All these factors have been considered in developing the following recommended water quality requirements for aquatic life.

It is the purpose of this document to define the water quality requirements which must be met to insure a favorable environment for fish, other aquatic life, and wildlife. This report will do this by identifying those aspects of water quality that are most important in the light of current knowledge and quantifying them where possible Where quantification is not yet possible, narrative guidelines will be offered There is no doubt that the water quality requirements contained herein must be reviewed periodically and updated in the light of additional and improved scientific data. The recommendations given in this report are considered to be satisfactory for aquatic life. In all instances where natural conditions fall outside the recommended ranges, this environment may be marginal and should not be changed in such a way as to make it more unfavorable

zones of passage

ANY BARRIER to migration and the free movement of the aquatic biota can be harmful in a number of ways. Such barriers block the spawning migration of anadromous and catadromus species Many resident species make local migrations for spawning and other purposes and any barrier can be detrimental to their continued existence. The natural tidal movement in estuaries and downstream movement of planktonic organisms and of aquatic invertebrates in flowing fresh waters are important factors in the re-population of areas and the general economy of the water. Any chemical or thermal barrier destroys this valuable source of food and creates unfavorable conditions below or above it

It is essential that adequate passageways be provided at all times for the movement or drift of the biota. Water quality criteria favorable to the aquatic community must be maintained at all times in these passageways. It is recognized, however, that certain areas of mixing are unavoidable. These create harmfully polluted areas and for this reason it is essential that they be limited in width and length and be provided only for mixing. The passage zone must provide favorable conditions and must be in a continuous stretch bordered by the same bank for a considerable distance to allow safe and adequate passage up and down the stream, reservoir, lake, or estuary for free-floating and drift organisms.

The width of the zone and the volume of flow in it will depend on the character and size of the stream or estuary. Area, depth, and volume of flow must be sufficient to provide a usable and desirable passageway for fish and other aquatic organisms. Further, the cross-sectional area and volume of flow in the passageway will largely determine the percentage of survival of drift organisms. Therefore, the passageway should contain preferably 75 percent of the cross-sectional area and/or volume of flow of the stream or estuary. It is evident that where there are several mixing areas close together they should all be on the same side so the passageway is continuous. Concentrations of waste materials in passageways should meet the requirements for the water.

The shape and size of mixing areas will vary with the location, size, character, and use of the receiving water and should be established by proper administrative authority. From the standpoint of the welfare of the aquatic life resource, however, such areas should be as small as possible and be provided for mixing only Mixing should be accomplished as quickly as possible through the use of devices which insure that the waste is mixed with the allocated dilution water in the smallest possible area. At the border of this area, the water quality must meet the water quality requirements for that area. If, upon complete mixing with the available dilution water these requirements are not met, the waste must be pretreated so they will be met. For the protection of aquatic life resources, mixing areas must not be used for, or considered as, a substitute for waste treatment, or as an extension of, or substitute for, a waste treatment facility.

summary and key criteria

RECOMMENDATIONS given below are considered to be satisfactory for aquatic life. In all instances where natural conditions fall outside the recommended ranges, these conditions may be marginal and should not be changed in such a way as to make them more unfavorable

Freshwater organisms

Dissolved Materials

(1) Dissolved materials that are relatively innocuous; i e , their harmful effect is due to osmotic effects at high concentrations, should not be increased by more than one-third of the concentration that is characteristic of the natural condition of the subject water. In no instance should the concentration of total dissolved materials exceed 50 milliosmoles (the equivalent of 1500 mg/l NaCl)

(2) Dissolved materials that are harmful in relatively low concentrations are discussed in the section "Toxicity."

pH, Alkalinity, Acidity

(1) No highly dissociated materials should be added in quantities sufficient to lower the pH below 6.0 or to raise the pH above 9.0.

(2) To protect the carbonate system and thus the productivity of the water, acid should not be added in sufficient quantity to lower the total alkalinity to less than 20 mg/l.

(3) The addition of weakly dissociated acids and alkalies should be regulated in terms of their own toxicities as established by bioassay procedures.

Temperature

Warm Water Biota: To maintain a well-rounded population of warm-water fishes, the following restrictions on temperature extremes and temperature increases are recommended:

(1) During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water (at the expected minimum daily flow for that month) more than 5 F. In lakes, the temperature of the epilimnion in those areas where important organisms are most likely to be adversely affected should not be raised more than 3 F above that which existed before the addition of heat of artificial origin The increase should be based on the monthly average of the maximum daily temperature Unless a special study shows that a discharge

of a heated effluent into the hypolimnion will be desirable, such practice is not recommended and water for cooling should not be pumped from the hypolimnion to be discharged to the same body of water.

(2) The normal daily and seasonal temperature variations that were present before the addition of heat due to other than natural causes should be maintained.

(3) The recommended maximum temperatures that are not to be exceeded for various species of warm-water fish are given in table III-1.

Cold Water Biota: Because of the large number of trout and salmon waters which have been destroyed, made marginal, or nonproductive, remaining trout and salmon waters must be protected if this resource is to be preserved.

Inland trout streams, headwaters of salmon streams, trout and salmon lakes, and the hypolimnion of lakes and reservoirs containing salmonids and other cold water forms should not be warmed or used for cooling water. No heated effluents should be discharged in the vicinity of spawning areas.

For other types and reaches of cold-water streams, reservoirs and lakes, the following restrictions are recommended:

(1). During any month of the year heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month). In lakes, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin

(2) The normal daily and seasonal temperature fluctuations that existed before the addition of heat due to other than natural causes should be maintained

TABLE III–1

[Provisional maximum temperatures recommended as compatible with the well-being of various species of fish and their associated biota]

93 F: Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, and gizzard shad.
90 F: Growth of largemouth bass, drum, bluegill, and crappie.
84 F: Growth of pike, perch, walleye, smallmouth bass, and sauger.
80 F: Spawning and egg development of catfish, buffalo, threadfin shad, and gizzard shad
75 F. Spawning and egg development of largemouth bass, white and yellow bass, and spotted bass.
68 F: Growth or migration routes of salmonids and for egg development of perch and smallmouth bass.
55 F: Spawning and egg development of salmon and trout (other than lake trout).
48 F: Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon.

Note —Recommended temperatures for other species, not listed above, may be established if and when necessary information becomes available

(3) The recommended maximum temperatures that are not to be exceeded for various species of cold-water fish are given in table III–1.

Dissolved Oxygen

The following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life.

(1) For a diversified warm-water biota, including game fish, DO concentration should be above 5 mg/l, assuming normal seasonal and daily variations are above this concentration. Under extreme conditions, however, they may range between 5 and 4 mg/l for short periods during any 24-hour period, provided that the water quality is favorable in all other respects. In stratified lakes, the DO requirements may not apply to the hypolimnion. In shallow unstratified lakes, they should apply to the entire circulation water mass.

These requirements should apply to all waters except administratively established mixing zones. In lakes, such zones must be restricted so as to limit the effect on the biota. In streams, there must be adequate and safe passageways for migrating forms. These must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage).

(2) For the cold-water biota, it is desirable that DO concentrations be at or near saturation. This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time. For good growth and the general well-being of trout, salmon, and their associated biota, DO concentrations should not be below 6 mg/l. Under extreme conditions, they may range between 6 and 5 mg/l for short periods provided the water quality is favorable in all other respects and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may range between 4 and 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place.

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding waste or other materials.

Carbon Dioxide

According to our present knowledge of the subject, it is recommended that the "free" carbon dioxide concentration should not exceed 25 mg/l.

Oil

Oil or petrochemicals should not be added in such quantities to the receiving waters that they will—

(1) produce a visible color film on the surface;
(2) impart an oily odor to the water or an oily or other noxious taste to fish and edible invertebrates;
(3) coat the banks and bottoms of the water course or taint any of the associated biota;
(4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

(1) Turbidity in the receiving waters due to the discharge of wastes should not exceed 50 Jackson units in warm-water streams or 10 Jackson units in cold-water streams.

(2) There should be no discharge to warm-water lakes which would cause turbidities exceeding 25 Jackson units The turbidity of cold-water or oligotrophic lakes should not exceed 10 units.

Settleable Materials

Since it is known that even minor deposits of settleable materials inhibit the growth of normal stream and lake flora, no such materials should be added to these waters in quantities that adversely affect the natural biota.

Color and Transparency

For effective photosynthetic production of oxygen, it is required that 10 percent of the incident light reach the bottom of any desired photosynthetic zone in which adequate dissolved oxygen concentrations are to be maintained

Floating Materials

All floating materials of foreign origin should be excluded from streams and lakes.

Tainting Substance

All materials that will impart odor or taste to fish or edible invertebrates should be excluded from receiving waters at levels that produce tainting.

Radionuclides

(1) No radioactive materials should be present in natural waters as a consequence of the failure of an installation to exercise appropriate controls to minimize releases.

(2) No radionuclide or mixture of radionuclides should be present at concentrations greater than those specified by the USPHS Drinking Water Standards.

(3) The concentrations of radioactive materials present in fresh, estuarine, and marine waters should be less than those that would require restrictions on the use of organisms harvested from the area to meet the Radiation Protection Guides recommended by the Federal Radiation Council.

Plant Nutrients and Nuisance Growths

The Subcommittee wishes to stress that the concentrations set forth are suggested solely as guidelines and the maintenance of these may or may not prevent undesirable blooms. All the factors causing nuisance plant growth and the level of each which should not be exceeded are not known.

(1) In order to limit nuisance growths, the addition of all organic wastes such as sewage, food processing, cannery, and industrial wastes containing nutrients, vitamins, trace elements, and growth stimulants should be carefully controlled. Furthermore, it should be pointed out that the addition of sulfates or manganese oxide to a lake should be limited if iron is present in the hypolimnion as they may increase the quantity of available phosphorus

(2) Nothing should be added that causes an increased zone of anaerobic decomposition of a lake or reservoir.

(3) The naturally occurring ratios and amounts of nitrogen (particularly NO_3 and NH_4) to total phosphorus should not be radically changed by the addition of materials As a guideline, the concentration of total phosphorus should not be increased to levels exceeding 100μg/l in flowing streams or 50 μg/l where streams enter lakes or reservoirs.

(4) Because of our present limited knowledge of conditions promoting nuisance growth, we must have a biological monitoring program to determine the effectiveness of the control measures put into operation. A monitoring program can detect in their early stages the development of undesirable changes in amounts and kinds of rooted aquatics and the condition of algal growths. With periodic monitoring, such undesirable trends can be detected and corrected by more stringent regulation of added organics

Toxic Substances

(1) **Substances of Unknown Toxicity:** All effluents containing foreign materials should be considered harmful and not permissible until bioassay tests have shown otherwise It should be the obligation of the agency producing the effluent to demonstrate that it is harmless in the concentrations to be found in the receiving waters All bioassays should be conducted strictly as recommended in the body of this report and the appropriate appli-

cation factor applied to determine the permissible concentration of toxicant.

(2) **Pesticides.**

(a) **Chlorinated hydrocarbons:** Any addition of chlorinated hydrocarbon insecticides is likely to cause damage to some desired organisms and should be avoided.

(b) **Other chemical pesticides:** Addition of other kinds of chemicals used as pesticides and herbicides can cause damage to desirable organisms and should be applied with utmost discretion and caution. Table III–5 (p. 62) lists the 48-hour TL_m values of a number of pesticides for various types of fresh water organisms. To provide reasonably safe concentrations of these materials in receiving waters, application factors ranging from $1/10$ to $1/100$ should be used with these values depending on the characteristic of the pesticide in question and used as specified in (4), below. Concentrations thus derived may be considered tentatively safe under the conditions specified.

(3) **Other Toxic Substances.**

(a) **ABS:** Concentration of continuous exposure to ABS should not exceed $1/7$ of the 48-hour TL_m A concentration as high as 1 mg/l may be tolerated occasionally for periods of time not exceeding 24 hours. ABS may increase the toxicity of other materials.

(b) **LAS:** The concentration of LAS should not exceed 0.2 mg/l or $1/7$ of the 48-hour TL_m.

(4) **Application Factors:** Concentration of materials that are nonpersistant (that is, have a half-life of less than 96 hours) or have noncumulative effects after mixing with the receiving waters should not exceed $1/10$ of the 96-hour TL_m value at any time or place. The 24-hour average of the concentration of these materials should not exceed $1/20$ of the TL_m value after mixing. For other toxicants the concentrations should not exceed $1/20$ and $1/100$ of the TL_m value under the conditions described above. Where specific application factors have been determined, they will be used in all instances.

(5) **General Considerations.** When two or more toxic materials that have additive effects are present at the same time in the receiving water, some reduction is necessary in the permissible concentrations as derived from bioassays on individual substances or wastes. The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the derived permissible concentration. An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible concentration for the mixture is through the use of following relationship:

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b}\ \cdots\ +\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b, . . . C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b, . . L_n are the respective permissible concentration limits derived for the materials on an individual basis. Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary.

Marine and estuarine organisms

Salinity

To protect estuarine organisms, no changes in channels, basin geometry, or freshwater influx should be made which would cause permanent changes in isohaline patterns of more than 10 percent of the naturally occurring variation.

Currents

Currents are important for transporting nutrients, larvae, and sedimentary materials for flushing and purifying wastes, and for maintaining patterns of scour and fill. To protect these functions, there should be no changes in basin geometry or freshwater inflow that will alter current patterns in such a way as to adversely affect existing biological and sedimentological situations

pH

No materials that extend normal ranges of pH at any location by more than 0.1 pH unit should be introduced into salt water portions of tidal tributaries or coastal waters. At no time should the introduction of foreign materials cause the pH to be less than 6.7 nor greater than 8.5.

Temperature

In view of the requirements for the well-being and production of marine organisms, it is concluded that the discharge of any heated waste into any coastal or estuarine waters should be closely managed. Monthly means of the maximum daily temperatures recorded at the site in question and before the addition of any heat of artificial origin should not be raised by more than 4 F during the fall, winter, and spring (September through May), or by more than 1.5 F during the summer (June through August), North of Long Island and in the waters of the Pacific Northwest (north of California), summer limits apply July through September; and fall, winter, and spring limits apply October through June. The rate of temperature change should not exceed 1 F per hour except when due to natural phenomena.

Suggested temperatures are to prevail outside of established mixing zones as discussed in the section on zones of passage.

Dissolved Oxygen

Oxygen levels sufficient for the survival, growth, reproduction, general well-being, and production of a suitable crop must be maintained. The dissolved oxygen concentrations necessary to attain this objective in coastal waters, estuaries, and tidal tributaries are:

(1) Dissolved oxygen concentrations in surface coastal waters should be greater than 5.0 mg/l except when upwellings and other natural phenomena may cause this value to be depressed.

(2) Dissolved oxygen concentration in estuaries and tidal tributaries should not be less than 4.0 mg/l at any time or place except in naturally dystrophic waters or where natural conditions cause DO to be depressed.

Oil

No oil or petroleum products should be discharged into estuarine or coastal waters in quantities that: (1) Can be detected as a visible film, sheen, or by odor; (2) cause tainting of fish or edible invertebrates; (3) form an oil sludge deposit on the shores or bottom of the receiving body of water, (4) become effective toxicants according to the criteria recommended in the "Toxicity" section.

Turbidity

No effluent that may cause changes in turbidity or color should be allowed to enter estuarine or coastal waters unless it can be shown to have no deleterious effects on the aquatic biota.

Settleable and Floating Substances

No materials that contain settleable solids or substances that may precipitate out in quantities that adversely affect the biota should be introduced into coastal or estuarine waters It is especially urgent that areas which serve as habitat or nursery grounds for commercially important species be protected from any impairment of natural conditions.

Tainting Substances

Substances that taint or produce off-flavors in fish and edible invertebrates should not be present in concentrations discernible by bioassay or organoleptic tests

Radionuclides

The recommendations made for freshwater organisms apply to marine and estuarine organisms.

Plant Nutrients and Nuisance Organisms

(1) No changes should be made in the basin geometry, current structure, salinity, or temperature of the estuary until studies have shown that these changes will not adversely affect the biota or promote the increase of nuisance organisms.

(2) The artificial enrichment of the marine environment from all sources should not cause any major quantitative or qualitative alteration in the flora such as the production of persistant blooms of phytoplankton (whether toxic or not), dense growths of attached algae or higher aquatics, or any other sort of nuisance that can be attributed directly to nutrient excess or imbalance Because these nutrients often are derived largely from drainage from land, special attention should be given to correct land management in river basins and shores of embayments to control unavoidable erosion.

(3) The naturally occurring atomic ratio of NO_3-N to PO_4-P in a body of water should be maintained. Similarly, the ratio of inorganic phosphorus (orthophosphate) to total phosphorus (the sum of inorganic phosphorus, dissolved organic phosphorus, and particulate phosphorus) should be maintained as it occurs naturally. Nutrient imbalances have been shown to cause a change in the natural diversity of desirable organisms and to reduce productivity

Toxic Substances

(1) **Substances of Unknown Toxicity:** All effluents containing foreign materials should be considered harmful and not permissible until bioassay tests have shown otherwise. It should be the obligation of the agency producing the effluent to demonstrate that it is harmless in the concentrations that will be found in the receiving waters. All bioassays should be conducted strictly as recommended in the body of this report and the appropriate application factor applied to determine the permissible concentration of toxicant.

(2) **Pesticides for Which Limits Have Been Determined:** The pesticides are grouped according to their relative toxicity to shrimp Criteria are based on the best estimates in the light of present knowledge and it is to be expected that acceptable levels of toxic materials may be changed as a result of future research.

Pesticide group A.—The following chemicals are acutely toxic at concentrations of 5 μg/l and less. On the assumption that $\frac{1}{100}$ of this level represents a reasonable application factor, it is recommended that environmental levels of these substances not be permitted to rise above 50 nanograms/l. This level is so low that these pesticides could not be applied directly in or near the marine habitat without danger of causing damage. The 48-hour TL_m is listed for each chemical in μg/l.

Organochloride pesticides

Aldrin	0.04	DDT	0.6
BHC	2.0	Dieldrin	0.3
Chlordane	2.0	Endosulfan	0.2
Endrin	0.2	Methoxychlor	4.0
Heptachlor	0.2	Perthane	3.0
Lindane	0.2	TDE	3.0
		Toxaphene	3.0

Organophosphorus pesticides

Coumaphos	2.0	Naled	3.0
Dursban	3.0	Parathion	1.0
Fenthion	0.03	Ronnel	5.0

Pesticide group B.—The following types of pesticide compounds are generally not acutely toxic at levels of 1.0 mg/l or less. It is recommended that an application factor of $\frac{1}{100}$ be used and in the absence of acute toxicity data that an environmental level of not more than 10 μg/l be permitted. An acute toxicity factor must be established for each specific chemical in this group to determine that it is not more toxic than related compounds as indicated above:

Arsenicals	2,4,5-T compounds
Botanicals	Phthalic acid compounds
Carbamates	Triazine compounds.
2,4-D compounds	Substituted urea compounds.

Other Pesticides.—Acute toxicity data are available for approximately 100 technical-grade pesticides in general use not listed in the above groups. These chemicals are either not likely to reach the marine environment or, if used as directed by the registered label, probably would not occur at levels toxic to marine biota. It is presumed that criteria established for these chemicals in fresh water will protect adequately the marine habitat. It should be emphasized that no unlisted chemical should be discharged into the estuary without preliminary bioassay tests.

(3) **Industrial and Other Toxic Wastes.**

(a) Safe concentrations of metals, ammonia, cyanide, and sulfide should be determined by the use of appropriate application factors to 96-hour TL_m values as determined by flow-through bioassays using dilution water that came from the receiving body. Test organisms should be local species or life stages of organisms of economic and ecologic importance which are the most sensitive to the waste in question. Application factors should be $\frac{1}{100}$ for metals, $\frac{1}{20}$ for ammonia, $\frac{1}{10}$ for cyanide, and $\frac{1}{20}$ for sulfide.

(b) Fluoride concentrations should not exceed those for drinking water.

(c) Permissible levels of detergents in fresh waters should also be applied to the marine and estuarine waters

(d) Bacteriological criteria of estuarine waters utilized for shellfish cultivation and harvesting should conform with the standards as described in the National Shellfish Sanitation Program Manual of Operation. These standards provide that—

(*1*) examinations shall be conducted in accordance with the American Public Health Association recommended procedures for the examination of sea water and shellfish,

(*2*) there shall be no direct discharges of untreated sewage,

(*3*) samples of water for bacteriological examination to be collected under those conditions of time and tide which produce maximum concentration of bacteria;

(*4*) the coliform median MPN of the water does not exceed 70/100 ml, and not more than 10 percent of the samples ordinarily exceed an MPN of 230/100 ml for a five-tube decimal dilution test (or 330/100 ml, where the three-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions;

(*5*) the reliability of nearby waste treatment plants shall be considered in the approval of areas for direct harvesting.

(e) Wastes from tar, gas, coke, petrochemical, pulp and paper manufacturing, waterfront and boating activities, hospitals, marine laboratories and research installation wastes are all complex mixtures having great variability in character and toxicity. Due to this variability, safe levels must be determined at frequent intervals by flow-through bioassays of the individual effluents.

For those operations having persistent toxicants, an application factor of $\frac{1}{100}$ should be used while for those composed largely of unstable or biodegradable toxicants, an application factor of $\frac{1}{20}$ is tentatively suggested.

(4) **General Considerations.**—When two or more toxicants that have additive effects are present, they must be treated as suggested earlier under fresh water organisms.

Wildlife

Dissolved Oxygen

In addition to the DO requirements for aquatic organisms, the bottoms of areas used by wildfowl must be kept aerobic to suppress botulinus organisms.

pH

Aquatic plants of greatest value as food for waterfowl thrive best in waters with a summer pH range of 7.0 to 9 2.

Alkalinity

Waterfowl habitats, to be productive, should have a bicarbonate alkalinity between 30 and 130 mg/l. Fluctuations should be less than 50 mg/l from natural conditions.

Salinity

Salinity should be kept as close to natural conditions as possible. Fluctuations in salinity during any 24-hour period should be limited as follows.

Natural salinity:	*Variation permitted*
0 to 3 5‰	1‰
3 5 to 13.5‰	2‰
13 5 to 35 0‰	4‰

Light Penetration

Optimum light requirements for aquatic wildlife habitats should be at least 10 percent of incident light at the surface to a 6-foot depth; the tolerable limit should be 5 percent of the light at the surface to the same depth.

Settleable Substances

Settleable substances destroy the usefulness of aquatic bottoms for waterfowl. Settleable substances should be excluded from areas expected to support waterfowl.

Oil

Oil is an especially dangerous substance to waterfowl. Oil and petrochemicals must be excluded from both the surface and bottoms of any area used by waterfowl.

Toxic Substances

Toxic substances should be excluded from wildlife habitats to the degree that they affect the health and well-being of wildlife, either directly or through biological magnification. Special consideration must be given to keep edible wildlife safe for consumption by humans

Disease

Offal from poultry houses, meatpacking plants, as well as other possible sources of disease organisms, must be excluded from areas supporting wildlife to guard against transmission of such diseases as botulism, fowl cholera, and aspergillosis.

General

Water quality suitable for fish and other aquatic organisms will be adequate for wildlife.

fresh water organisms

Dissolved materials

Water devoid of dissolved materials is intolerable in nature because pure water will not support aquatic life. Natural waters contain endless varieties of dissolved materials in concentrations that differ widely from one locality to another as well as from time to time. Many of these dissolved materials are essential for growth, reproduction, and the general well-being of aquatic organisms. The chlorides, carbonates, and silicates of sodium, potassium, calcium, and magnesium are generally the most common salts present Traces of most other essential substances are also found.

Aquatic organisms live in different concentrations of dissolved substances but productivity declines as the concentrations move away from the optimum Seldom, if ever, are the dissolved substances at the optimum concentrations as we know them The range of tolerance may be relatively wide, but when the concentrations reach too low or too high a level, organisms degenerate and die Different organisms vary in their optimum requirements as well as in their ability to live and thrive under variations from the optimum Some organisms are equally at home in sea water and in fresh water. Other organisms will tolerate only one or the other.

Any of the substances necessary to aquatic organisms has a range of concentration that is both essential and tolerable The tolerance levels for any one substance vary depending on the concentrations of other substances present. The presence of certain substances synergizes the effects of some materials but antagonizes the effects of others. Under optimal concentrations, the synergistic and antagonistic effects are in balance and relatively high concentrations can be tolerated without adverse effects

Although several measures of dissolved materials are available, no measure in itself is adequate as an index of optimum concentration nor is any single measure adequate to express the range of tolerance. The biological effects depend on the concentrations of the individual solutes, some of which are tolerated in terms of grams per liter but others only in nanograms per liter Some exert considerable osmotic pressure, but for others the osmotic effect is negligible. Some substances contribute greatly to conductivity, while others have little or no effect

In general, the concentrations of dissolved materials in natural fresh waters are below the optimum for maximum productivity In many instances, therefore, the addition of any of a large number of substances will be beneficial. In this way, many water courses have a capacity to absorb materials to advantage. But the addition of what may be considered beneficial substances must be controlled so that they will not exceed favorable limits.

The osmotic concentration of the body fluids of a fresh water animal is generally the maximum concentration of dissolved material that the animal will tolerate In some animals, notably some of the fresh water mollusks, the body fluids have an osmotic concentration as low as 50 milliosmoles (the equivalent of about 0 025 molar or 1,500 mg/l sodium chloride) If the dissolved materials are relatively innocuous, having only an osmotic effect, it is judged that the total dissolved materials in a water course may be increased to a certain extent but they should not exceed 50 milliosmoles if the fauna is to be maintained.

Many species of diatoms are very sensitive to changes in chloride and other salt concentrations. Some species, such as those in mountain streams and in black water streams of the coastal plains, can live only in waters with extremely low concentrations of salts. The addition of salts to such streams will eliminate many desirable species of diatoms and permit undesirable species to flourish Such changes may reduce the desirable food sources and bring about nuisance problems as well It is believed that the total dissolved material in a water course should not be increased by more than one-third of that which is characteristic of the natural conditions of such a water course

The toxicity of substances added to natural waters often depends on the substances already present in the receiving waters With synergism, the toxicity increases, and with antagonism it decreases. Again the reaction of the toxic substances may produce, in some cases, new products of greater toxicity, and in others, products of lesser toxicity

In view of the many factors that become involved in the disposal of soluble materials in natural waters, it is evident that no simple answer is available. Therefore, bioassays should be used to determine the amounts of the materials that may be tolerated without reducing the productivity of the water course in question

Recommendation: Dissolved materials are of two types: those that are toxic at very low concentrations and those, such as the salts of the earth metals, that are required in certain concentrations for a productive water and become harmful only at high concentrations by exerting an osmotic effect If the dissolved materials are relatively innocuous, i e, their harmful effect is an osmotic one at high concentrations, it is judged that the total dissolved materials of this type may be increased to a certain extent but they should not exceed 50 milliosmoles in waters where diversified animal populations are to be protected Further, to maintain local conditions, total dissolved materials should not be increased by more than one-third of the concentration that is characteristic of the natural condition of the water When dissolved materials are being increased, bioassays and field studies should be used to determine how much of the materials may be tolerated without reducing the productivity of the desired organisms.

Acidity alkalinity, and pH

Acidity and alkalinity are reciprocal terms Acidity is produced by substances that yield hydrogen ions on hydrolysis and alkalinity is produced by substances that yield hydroxyl ions. Other definitions state that a substance is acid if it will neutralize hydroxyl ions and a substance is alkaline if it will neutralize hydrogen ions. The terms "total acidity" and "total alkalinity" are often used to express the buffering capacity of a solution Acidity in natural waters is caused by carbon dioxide, mineral acids, weakly dissociated acids, and the salts of strong acids and weak bases. Alkalinity is caused by strong bases and the salts of strong alkalies and weak acids.

An index of the hydrogen ion activity is pH. Even though pH determinations are used as an indication of acidity and/or alkalinity, pH is not a measure of either As pointed out in the first sentence in the previous paragraph, acidity and alkalinity are reciprocal terms Indeed, a water may have both an acidity and alkalinity at the same time. Total acidity, by definition, is the amount of standard alkali required to bring a sample to pH 8 3. Total alkalinity, similarly, is the amount of standard acid required to bring a sample to pH 4 5 Both are expressed in equivalents of $CaCO_3$. Under these circumstances, there is a relationship between pH, acidity, and alkalinity since, by definition (see Standard Methods for the Examination of Water and Wastewater, 12th edition 1965), any water with a pH of 4.5 or lower has no measurable alkalinity and a water with a pH of 8 3 or higher has no measurable acidity.

In natural waters, where the pH is in the vicinity of 8 3, acidity is not a factor of concern. In most productive, fresh, natural waters, the pH falls in the range between 6.5 and 8.5 (except when increased by photosynthetic activity). Some aquatic organisms have been found to live at pH 2 and lower and others at pH 10 and higher; however, such organisms are relatively few. Some natural waters with a pH of 4 support fish and other organisms. In these cases the acidity is due primarily to carbon dioxide and humic acids and the water has little buffering capacity (low total alkalinity). Other natural waters with a pH of 9.5 also support fish, but in such situations the waters are not regarded as highly productive.

Acids that dissociate to a high degree do not appear to be toxic at pH values above 6.0. They are toxic if added in sufficient quantities to reduce the pH to less than 6.0 Acids that dissociate to a low degree are often toxic at pH values considerably above 6.0. In the latter condition, toxicity is due either to the anion or to the compound itself; e.g., hydrogen cyanide (HCN), hydrogen sulfide (H_2S), and hypochlorous (HClO) and tannic acids.

Alkalies that dissociate to a high degree do not appear to be toxic at pH values below 9.0 Alkaline compounds that dissociate to a low degree are often toxic at pH values less than 9.0 and their toxicity is due either to the cation or to the undissociated molecule. Ammonium hydroxide is an example. Temporarily high pH levels often are produced in highly productive waters through photosynthetic activity of the aquatic plants by converting the carbonate to the hydroxide, which results in an increased pH. Because these high pH levels prevail for only a few hours, they do not produce the harmful effects of continuous high levels due to the presence of strong alkalies.

Addition of either acids or alkalies to waters may be harmful not only in producing adverse acid or alkaline conditions, but also by increasing the toxicity of various components in the waters. The addition of strong acids may cause the formation of carbonic acid (free CO_2) in quantities that are adverse to the well-being of the organisms present. A reduction of about 1 5 pH units can cause a thousand-fold increase in the acute toxicity of a metallo-cyanide complex. The addition of strong alkalies may cause the formation of undissociated NH_4OH or un-ionized NH_3 in quantities that may be toxic The availability of many nutrient substances varies with the acidity and alkalinity. At higher pH values, iron tends to become unavailable to some plants.

The nonlethal limits of pH are narrower for some fish food organisms than they are for fish. For example, *Daphnia magna* does not survive experimentally in water having a pH below 6 0

The major buffering system in natural waters is the carbonate system. This system not only neutralizes acids and bases so as to reduce the fluctuations in pH, but also forms an indispensable reservoir of carbon for photosynthesis, because there is a decided limit on the rate at which carbon dioxide can be obtained from the atmosphere to replace that in the water which becomes fixed by the plants. Thus the productivities of waters are closely correlated with the carbonate buffering systems. The addition of mineral acids preempts the carbonate buffering capacity and the original biological productivity is reduced in proportion to the degree that such capacity is exhausted. It is as necessary, therefore, to maintain the minimum essential buffering capacity as it is to confine the pH of the water within tolerable limits.

Recommendation: (1) In view of the above considerations and their importance for the production and well-being of aquatic organisms, no highly dissociated materials should be added in quantities sufficient to lower the pH below 6 0 or to raise the pH above 9 0.

(2) To protect the carbonate system and thus the productivity of the water, acid should not be added sufficient to lower the total alkalinity below 20 mg/l expressed as $CaCO_3$

(3) The addition of weakly dissociated acids and alkalies should be regulated in terms of their own toxicities as established by bioassay procedures

Hardness

Hardness was originally considered as the capacity of water to precipitate or neutralize soap. In natural waters, hardness is chiefly attributable to calcium and magnesium ions. Other ions, such as strontium, barium, aluminum, manganese, iron, copper, zinc, and lead also are responsible for hardness, but since they are present in relatively minor concentrations, their role usually can be ignored. Hardness, like acidity and alkalinity, is expressed in terms of $CaCO_3$ but the hardness of a water is not necessarily equal to either the acidity or alkalinity Hardness in natural waters is generally correlated with dissolved solids but there are exceptions

Generally, the biological productivity of a water is directly correlated with its hardness, but hardness *per se* has no biological significance because productivity depends on the specific combination of elements present. Calcium and magnesium contribute to hardness and to productivity. Most other elements that contribute to hardness reduce biological productivity and are toxic when they produce a substantial measure of hardness. Because hardness of itself has no biological significance, and because some elements which contribute to hardness may enhance biological productivity (while other contributing elements are toxic), it is recommended that the term hardness be avoided in dealing with water quality requirements for aquatic life

Temperature

The relationships of temperature and aquatic life have been well studied Extensive bibliographies and detailed surveys of the subject have been published by the American Society of Civil Engineers (1967), Brett (1960), Mihursky and Kennedy (1967), Raney (1966), U.S Department of Interior, Federal Water Pollution Control Administration (1967), and Wurtz and Renn (1965)

The temperatures of the surface waters of the United States vary from 32 to over 100 F as a function of latitude, altitude, season, time of day, duration of flow, depth, and many other variables The agents that may affect the natural temperature are so numerous that it seems unlikely that two bodies of water, even in the same latitude, would have exactly the same thermal characteristics The fish and other aquatic life occurring naturally in each body of water are species or varieties that are competing there with various degrees of success depending on the temperature and various other conditions existing in that habitat This adaptation extends not only to temperature and the range over which it can vary, but also to such factors as day length and the other species of animals and plants in the same habitat. The interrelationships of species, day length, and water temperature are so intimate that even a small change in temperature may have far-reaching effects An insect nymph in an artificially warmed stream, for example, might emerge for its mating flight too early in the spring and be immobilized by the air temperature Similarly, a fish might hatch too early in the spring to find an adequate amount of its natural food organisms because the food chain depends ultimately on plants whose abundance in turn, is a function of day length and temperature The inhabitants of a water body that seldom becomes warmer than 70 F are placed under stress, if not killed outright, by 90 F water Even at 75 to 80 F, they may be unable to compete successfully with organisms for which 75 to 80 F is a favorable temperature. Similarly, the inhabitants of warmer waters are at a competitive disadvantage in cool water.

Although in a rigorous climate, an animal can endure the extremes of temperature at appropriate seasons; it must be cooled gradually in the fall if it is to become acclimatized to the cold water of winter and warmed gradually in the spring if it is to withstand summer heat Further, an organism might be able to endure a high temperature of 92 or 95 F for a few hours, but it could not do so for a period of days. Having the water change gradually with the season is important for other reasons. an increasing or decreasing temperature often serves as the trigger for spawning activities, metamorphosis, and migration Some fresh water organisms require that their eggs be chilled before they will hatch properly.

In arriving at suitable temperature criteria, the problem is to estimate how far the natural temperature may be exceeded without adverse effects Whatever requirements are suggested, a seasonal cycle must be retained, the changes in temperature must be gradual and the temperature reached must not be so high or so low as to damage or alter the composition of the desired population. In view of the many variables, it seems obvious that no single temperature requirement can be applied to the United States as a whole, or even to one State; the requirements must be closely related to each body of water and its population To do this a temperature increment based on the natural water temperature is more appropriate than an unvarying number Using an increment requires, however, that we have information on the natural temperature conditions of the water in question, and the size of the increment that can be tolerated by the desired species

If any appreciable heat load is introduced into a stream, it must be recognized that the species' equilibrium will likely be shifted towards that characteristic of a more southerly water

The seasonal temperature fluctuation normal to the desired biota of a particular water must be maintained Further, the sum of any increase in temperature plus the natural peak temperature should be of short duration and below the maximum temperature that is detrimental for such periods

Recommendation for Warm Waters: To maintain a well-rounded population of warm-water fishes, the following restrictions on temperature extremes and temperature increases are recommended

(1) During any month of the year, heat should not be added to a stream in excess of the amount that will raise the temperature of the water (at the expected minimum daily flow for that month) more than 5 F In lakes and reservoirs, the temperatures of the epilimnion, in those areas where important organisms are most likely to be adversely affected, should not be raised more than 3 F above that which existed before the addition of heat of artificial origin The increase should be based on the monthly average of the maximum daily temperature Unless a special study shows that a discharge of a heated effluent into the hypolimnion or pumping water from the hypolimnion (for discharging back into the same water body) will be desirable, such practice is not recommended

(2) The normal daily and seasonal temperature variations that were present before the addition of heat, due to other than natural causes, should be maintained

(3) The recommended maximum temperatures that are not to be exceeded for various species of warm-water fish are given in table III-1

Recommendation for Cold Waters: Because of the large number of trout and salmon waters which have been destroyed, or made marginal or nonproductive, the remaining trout and salmon waters must be protected if this resource is to be preserved.

(1) Inland trout streams, headwaters of salmon streams, trout and salmon lakes and reservoirs, and the hypolimnion of lakes and reservoirs containing salmonids should not be warmed. No heated effluents should be discharged in the vicinity of spawning areas.

For other types and reaches of cold-water streams, reservoirs, and lakes, the following restrictions are recommended

(2) During any month of the year, heat should not be added to a stream in excess of the amount that will raise the temperature of the water more than 5 F (based on the minimum expected flow for that month) In lakes and reservoirs, the temperature of the epilimnion should not be raised more than 3 F by the addition of heat of artificial origin.

(3) The normal daily and seasonal temperature fluctuations that existed before the addition of heat due to other than natural causes should be maintained

(4) The recommended maximum temperatures that are not to be exceeded for various species of cold water fish are given in table III–1

NOTE—For streams, total added heat (in BTU's) might be specified as an allowable increase in temperature of the minimum daily flow expected for the month or period in question This would allow addition of a constant amount of heat throughout the period. Approached in this way for all periods of the year, seasonal variation would be maintained. For lakes the situation is more complex and cannot be specified in simple terms.

TABLE III–1

[Provisional maximum temperatures recommended as compatible with the well-being of various species of fish and their associated biota]

93 F.	Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, and gizzard shad.
90 F·	Growth of largemouth bass, drum, bluegill, and crappie.
84 F:	Growth of pike, perch, walleye, smallmouth bass, and sauger.
80 F:	Spawning and egg development of catfish, buffalo, threadfin shad, and gizzard shad.
75 F:	Spawning and egg development of largemouth bass, white, yellow, and spotted bass
68 F:	Growth or migration routes of salmonids and for egg development of perch and smallmouth bass.
55 F:	Spawning and egg development of salmon and trout (other than lake trout)
48 F.	Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon.

Note—Recommended temperatures for other species, not listed above, may be established if and when necessary information becomes available

Dissolved oxygen

Oxygen requirements of aquatic life have been extensively studied. Excellent survey papers are presented by Doudoroff (1957), Doudoroff and Shumway (1967), Doudoroff and Warren (1962), Ellis (1937), and Fry (1960). Much of the work on temperature requirements also considers oxygen and those bibliographies are equally valuable.

Most of the research concerning oxygen requirements for freshwater organisms deals with fish, but since fish depend upon other aquatic species for food and would not remain in an area with an inadequate food supply, it seems reasonable to assume that a requirement for fish would serve also for the rest of the community The fish themselves can be grouped into three categories according to their temperature and oxygen requirements: (1) the cold-water fish (e.g, salmon and trout), (2) the warm-water game and pan fish (e.g., bass and sunfish), and (3) the warm-water "coarse" fish (e.g, carp and buffalo) The cold-water fish seem to require higher oxygen concentrations than the warm-water varieties. The reason is not known, but it may be related to the fact that, for half saturation, trout hemoglobin requires an oxygen partial pressure three or four times that required by carp hemoglobin under similar circumstances. Warm-water game and pan fish seem to require a higher concentration than the "coarse" fish, probably because the former are more active and predatory

Relatively little of the research on the oxygen requirements of fish in any of these three categories is applicable to the problem of establishing oxygen criteria because the endpoints have usually been too crude. It is useless in the present context to know how long an animal can resist death by asphyxiation at low dissolved oxygen concentrations; we must know instead the oxygen concentration that will permit an aquatic population to thrive. We need data on the oxygen requirements for egg development, for newly hatched larvae, for normal growth and activity, and for completing all stages of the reproductive cycle It is only recently that experimental work has been undertaken on the effects of oxygen concentration on these more subtle endpoints As yet, only a few species have been studied

One of the first signs that a fish is being affected by a reduction of dissolved oxygen (DO) concentration is an increase in the rate at which it ventilates its gills, a process accomplished in part by an increase in the frequency of the opercular movements The half dozen or so species (chiefly warm-water game and pan fish) that have been reported so far show a significant increase in frequency as the DO concentration is reduced from 6 to 5 mg/l (at about 72 F) and a greater increase from 5 to 4 mg/l. If the opercular rate is taken as the criterion by which the adequacy of an oxygen concentration is to be judged, then such evidence as we have indicates 6 mg/l as the required dissolved oxygen concentration Several field studies have shown, however, that good and diversified fish populations can occur in waters in which the dissolved oxygen concentration is between 6 and 5 mg/l in the summer, suggesting that a minimum of 6 mg/l is probably more stringent than necessary for warm-water fishes Because the oxygen content of a body of water does not remain constant, it follows that if the dissolved oxygen is never less than 5 mg/l it must be higher part of the time. In some cases, good populations of warm-water fish, including game and pan fishes, occur in waters in which the dissolved oxygen may be as low as 4 mg/l for short periods. Three mg/l is much too low, however, if normal growth and activity are to be maintained. It has been reported that the growth of young fish is slowed markedly if the oxygen concentration falls to 3 mg/l for part of the day, even if it rises as high as 18 mg/l at other times. It is for such reasons as this that oxygen criteria cannot be based on averages Five and 4 mg/l are close to the borderline of oxygen concentrations that are tolerable for extended periods. For a good population of game and pan fishes, the concentration should be considerably more than this.

The requirements of the different stages in the life cycles of aquatic organisms must be taken into account An oxygen concentration that can be tolerated by an adult animal, with fully developed respiratory apparatus, less intense metabolic requirements, and the ability to move away from adverse conditions, could easily be too low for eggs and larval stages The eggs are especially vulnerable to oxygen lack because they have to depend upon oxygen diffusing into them at a rate sufficient to maintain the developing embryos. Hatching, too, is a critical time; recently hatched young need relatively more oxygen than adults, but until they become able to swim for themselves (unless they are in flowing water) they must depend upon the oxygen supply in the limited zone around them These problems are not as great among species that tend their eggs and young, suspend their eggs from plants, or have pelagic eggs, as they are for salmonids Salmonids bury their eggs in the gravel of the stream away from the main flow of the water thereby requiring a relatively high oxygen concentration in the water that does reach them.

Recommendation: In view of the above considerations and with the proviso that future research may make revision necessary, the following environmental conditions are considered essential for maintaining native populations of fish and other aquatic life

(1) For a diversified warm-water biota, including game fish, daily DO concentration should be above 5 mg/l, assuming that there are normal seasonal and daily variations above this concentration Under extreme conditions, however, and with the same stipulation for seasonal and daily fluctuations, the DO may range between 5 mg/l and 4 mg/l for short periods of time, provided that the water quality is favorable in all other respects In stratified eutrophic and dystrophic lakes, the DO requirements may not apply to the hypolimnion In shallow unstratified lakes, they should apply to the entire circulating water mass

These requirements should apply to all waters except administratively established mixing zones In lakes, such mixing zones must be restricted so as to limit the effect on the biota In streams, there must be no blocks to migration and there must be adequate and safe passageways for migrating forms These zones of passage must be extensive enough so that the majority of plankton and other drifting organisms are protected (see section on zones of passage)

(2) For the cold water biota, it is desirable that DO concentrations be at or near saturation This is especially important in spawning areas where DO levels must not be below 7 mg/l at any time For good growth and the general well-being of trout, salmon, and other species of the biota, DO concentrations should not be below 6 mg/l Under extreme conditions they may range between 6 and 5 mg/l for short periods provided that the water quality is favorable and normal daily and seasonal fluctuations occur. In large streams that have some stratification or that serve principally as migratory routes, DO levels may be as low as 5 mg/l for periods up to 6 hours, but should never be below 4 mg/l at any time or place

(3) DO levels in the hypolimnion of oligotrophic small inland lakes and in large lakes should not be lowered below 6 mg/l at any time due to the addition of oxygen-demanding wastes or other materials

Carbon dioxide

An excess of "free" carbon dioxide (as distinguished from that present as carbonate and bicarbonate) may have adverse effects on aquatic animals These effects range from avoidance reactions and changes in respiratory movements at low concentrations, through interference with gas exchange at higher concentrations, to narcosis and death if the concentration is increased further The respiratory effects seem the most likely to be of concern in the present connection

Since the carbon dioxide resulting from metabolic processes leaves the organisms by diffusion, an increase in external CO_2 concentration will make it more difficult for it to diffuse out of the organism. Thus, it begins to accumulate internally The consequences of this internal accumulation are best known for fish, but presumably the principles are the same for other organisms. As the CO_2 accumulates, it depresses the blood pH, and this may have detrimental effects. Probably more important, however, is the fact that the greater the blood CO_2 concentration, the less readily will the animal's hemoglobin combine with dissolved oxygen Thus the presence of much CO_2 raises the minimum oxygen concentration which is tolerable Since the combination of oxygen with hemoglobin is inversely related to temperature, it is obvious that CO_2, temperature, and oxygen are closely related. Insufficient data are available at present to permit us to state the greatest amount of dissolved carbon dioxide that all types of aquatic organisms can tolerate and how these tolerable concentrations vary with temperature and dissolved oxygen. Studies of the effect of CO_2 on the oxygen requirements of several species of fish indicate that CO_2 concentrations of the order of 25 mg/l should not be detrimental, provided the oxygen concentration and temperature are within the recommended limits.

Recommendation: According to our rather meagre knowledge of the subject, it is recommended that the free CO_2 concentration should not exceed 25 mg/l

Oil

Oil slicks are barely visible at a concentration of about 25 gal/sq mi (Amer. Petroleum Inst. 1949). At 50 gal/sq mi, an oil film is 3.0×10^{-6} inches thick and is visible as a silvery sheen on the surface. Sources of oil pollution are bilge and ballast waters from ships, oil refinery wastes, industrial plant wastes such as oil, grease, and fats from the lubrication of machinery, reduction works, plants manufacturing hydrogenated glycerides, free fatty acids, and glycerine, rolling mills, county drains, storm-water overflows, gasoline filling stations, and bulk stations

Wiebe (1935) showed that direct contact by fish (bass and bream) with crude oil resulted in death caused by a film over the gill filaments. He also demonstrated that crude oil contains a water-soluble fraction that is very toxic to fish. Galtsoff, et al. (1935) showed that crude oil contains substances soluble in sea water that produce an anaesthetic effect on the ciliated epithelium of the gills of oysters Free oil and emulsions may act on the epithelial surfaces of fish gills and interfere with respiration. They may coat and destroy algae and other plankton, thereby removing a source of fish food, and when ingested by fish they may taint their flesh.

Setteable oily substances may coat the bottom, destroy benthic organisms, and interfere with spawning areas. Oil may be absorbed quickly by suspended matter, such as clay, and then due to wind action or strong currents may be transported over wide areas and deposited on the bottom far from the source Even when deposited on the bottom, oil continuously yields water-soluble substances that are toxic to aquatic life.

Films of oil on the surface may interfere with reaeration and photosynthesis and prevent the respiration of aquatic insects such as water boatmen, backswimmers, the larvae and adults of many species of aquatic beetles, and some species of aquatic Diptera (flies). These insects surface and carry oxygen bubbles beneath the surface by means of special setae which can be adversely affected by oil Berry (1951) reported that oil films on the lower Detroit River are a constant threat to waterfowl Oil is detrimental to waterfowl by destroying the natural buoyancy and insulation of their feathers

A number of observations made by various authors in this country and abroad record the concentrations of oil in fresh water which are deleterious to different species For instance, penetration of motor oil into a fresh water reservoir used for holding crayfish in Germany caused the death of about 20,000 animals (Seydell, 1913) It was established experimentally that crayfish weighing from 35 to 38 g die in concentrations of 5 to 50 mg/l within 18 to 60 hours Tests with two species of fresh water fish, ruff (small European perch), and whitefish (fam Coregonidae) showed that concentrations of 4 to 16 mg/l are lethal to these species in 18 to 60 hours.

The toxicity of crude oil from various oil fields in Russia varies depending on its chemical composition. The oil used by Veselov (1948) in the studies of the pollution of Belaya River (a tributary in the Kama in European Russia) belongs to a group of methano-aromatic oils with a high content of asphalt, tar compounds, and sulfur. It contains little paraffin and considerable amounts of benzene-ligroin. Small crucian carp (*Carassius carassius*) 7–9 cm long were used as the bioassay test animal This is considered to be a hardy fish that easily withstands adverse conditions. The water soluble fraction of oil was extracted by shaking 15 ml of oil in 1 liter of water for 15 minutes. The oil film was removed by filtration. Dissolved oxygen was controlled A total of 154 tests were performed using 242 fishes. The average survival time was 17 days at the concentration of 0 4 ml/l of oil but only 3 days at the concentration of 4 ml/l Further increase in concentration had no appreciable effect on fish mortality.

Seydell (1913) stated that the toxicity of Russian oil is due to naphthenic acids, small quantities of phenol, and volatile acids (Veselov, 1948).

Cairns (1957) reports the following 96-hour TL_m values of naphthenic acid for bluegill sunfish (*Lepomis macrochirus*)—5.6 mg/l; pulmonate snail (*Physa heterostropha*)—6 1 to 7 5 mg/l (in soft water), and diatom (species not identified)—41.8 to 43.4 mg/l in soft water and 28 2 to 79 8 mg/l in hard water. Naphthenic acid (cyclohexane carboxylic acid) is extracted from petroleum and is used in the manufacture of insecticides, paper, and rubber

Chipman and Galtsoff (1949) report that crude oil in concentrations as low as 0.3 mg/l is extremely toxic to fresh water fish. Dorris, Gould, and Jenkins (1960) made an intensive study of the toxicity of oil refinery effluents to fathead minnows in Oklahoma. By standard bioassay procedures, they found that mortality varied between 3.1 percent to 21.5 percent after 48 hours of exposure to untreated effluents. They concluded that toxicity rather than oxygen demand is the most important effect of oil refinery effluents on receiving streams.

Pickering and Henderson (1966b) reported the results of acute toxicity studies of several important petrochemicals to fathead minnows, bluegills, goldfish, and guppies in both soft water and hard water Standard bioassay methods were used Because several of the compounds tested have low solubility in water, stock solutions were prepared by blending the calculated concentrations into 500 ml of water before addition to the test container Where necessary, pure oxygen was supplied by bubbling at a slow rate The petrochemicals tested were benzene, chlorobenzene, 0-chlorophenol, 3-chloropropene, 0-cresol, cyclohexane, ethyl benzene, isoprene, methyl methacrylate, phenol, 0-phthalic anhydride, styrene, toluene, vinyl acetate, and xylene These petrochemicals are similar in their toxicities to fish, with 96-hour TL_m values ranging from 12 to 368 mg/l Except for isoprene and methyl methacrylate, which are less toxic, values for all four species of fish for the other petrochemicals ranged from 12 to 97 mg/l, a relatively small variation In general, 0-chlorophenol and 0-cresol are the most toxic and methyl methacrylate and isoprene are the least toxic

Recommendation: In view of available data, it is concluded that to provide suitable conditions for aquatic life, oil and petrochemicals should not be added in such quantities to the receiving waters that they will (1) produce a visible color film on the surface, (2) impart an oily odor to water or an oily taste to fish and edible invertebrates, (3) coat the banks and bottom of the water course or taint any of the associated biota, or (4) become effective toxicants according to the criteria recommended in the "Toxicity" section

Turbidity

Turbidity is caused by the presence of suspended matter such as clay, silt, finely divided organic matter, bacteria, plankton, and other microscopic oragnisms Turbidity is an expression of the optical property of a sample of water which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. Excessive turbidity reduces light penetration into the water and, therefore, reduces photosynthesis by phytoplankton organisms, attached algae, and submersed vegetation

The Jackson candle turbidimeter (Standard Methods for the Examination of Water and Wastewater, 12th edition 1965) is the standard instrument for making measurements of turbidity Field determinations, however, are made with direct-reading colorimeters calibrated for this test and the results are expressed as Jackson turbidity units (JTU)

Silt and sediment are particularly damaging to gravel and rubble-type bottoms The sediment fills the interstices between gravel and stones, thereby eliminating the spawning grounds of fish and the habitat of many aquatic insects and other invertebrate animals such as mollusks, crayfish, fresh water shrimp, etc Tarzwell (1957) observed that bottom organisms from a silted area averaged only 36 organisms/sq ft compared to 249/sq ft in a non-silted area Smith (1940) reported that silting reduced the bottom fauna of the Rogue River by 25 to 50 percent Observations in Oregon by Wagner (1959) and Ziebell (1960) showed an 85-percent decline in productivity of aquatic insect populations below a gravel dragline operation. Turbidities in the affected area were increased from zero to 91 mg/l and suspended solids from 2 mg/l upstream to 103 mg/l downstream

Buck (1956) investigated several farm ponds, hatchery ponds, and reservoirs over a 2-year period He observed that the maximum production of 161 5 lb/acre occurred in farm ponds where the average turbidity was less than 25 JTU. Between 25 and 100 JTU, fish yield dropped 41.7 percent to 94 lb/acre, and in muddy ponds, where turbidity exceeded 100 JTU, the yield was only 29 3 lb/acre or 18 2 percent of clear ponds

Herbert and Merkens (1961), using a mixture of kaolin and diatomaceous earth, demonstrated that long-term exposure of rainbow trout to 100–200 mg/l could be harmful At 270 and 810 mg/l, a high percentage of the fish died. Wallen (1951) studied the effects of montmorillonite clay on 16 species of warm-water fish. Results are shown in table III–2 It is shown that fish can tolerate high turbidities for short periods, a fortunate adaptation for river species Fish productivity is ultimately dependent upon plant life and a good bottom fauna. There can be little of either above 200 JTU if that turbidity is maintained continuously The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (ORSANCO) Second Progress Report (1956) points out that fish withstand turbidities of 5,000 mg/l or more with no direct harmful results, but the productivity of the bottom areas is very low and the fish populations are small

TABLE III–2. Average Turbidities Found To Be Fatal to Fish

Species	Length of exposure (days)	Turbidity (mg/l)
Large mouth bass	7 6	101,000
Pumpkin seed sunfish	13	69,000
Channel catfish	9 3	85,000
Black bullhead	17	222,000
Golden shiner	7 1	166,000

Ellis (1937) summarized the results of 2,344 light penetration determinations made at 585 stations on streams throughout the United States The determinations were made of the millionth intensity depth (m i d), which is the depth in millimeters of water of the given turbidity required to screen out 99 9999 percent of the light entering at the surface. A photoelectric apparatus described by Ellis (1934b) was used and determinations were made after filtering the water through bolting silk

The turbidity of rivers varies widely in different parts of the country Ellis (1937) defined clear streams as those with a m i d of 5 00 to infinity, cloudy streams, 4 90–1.00 meters, turbid, 0 99–0 50; very turbid, 0 49–0 30; muddy, 0 29–0 15, very muddy, 0 14–0 00 meters

In Mississippi River side channels and flowing stream tributaries with good fish fauna, 4 percent were clear, 11 percent cloudy, 3 percent were very muddy In these waters, with medium, poor, or no fish fauna 1 percent were clear, 18 percent cloudy, 11 percent turbid, 14 percent very turbid, 38 percent muddy, and 18 percent very muddy

Based on 6,000 light penetration determinations on inland streams, he concluded that, for good production of fish and aquatic life, the silt load of these streams should be reduced so that the millionth intensity depth would be greater than 5 meters.

Good farming practices can do a great deal to prevent silt from reaching streams and lakes. Road building and housing development projects, placer mining, strip mining, coal and gravel washing, and unprotected road cuts are important sources of turbidity that can be reduced with planning, good housekeeping, and regulation.

Natural turbidities within watersheds should be determined For example, in some Western States many streams have a turbidity below 25 JTU for most of the year In those states, the water pollution control agency might specify that no wastes should be discharged which would raise the turbidity of the receiving water above 25 JTU.

From the above discussion it can be seen that natural turbidity varies greatly in different parts of the country

Recommendation. Turbidity in the receiving water due to a discharge should not exceed 50 JTU in warm-water streams or 10 JTU in cold-water streams.

There should be no discharge to warm-water lakes which will cause turbidities exceeding 25 Jackson Units The turbidity of cold-water or oligotrophic lakes should not exceed 10 units

Settleable solids

Settleable solids include both inorganic and organic materials The inorganic components include sand, silt, and clay originating from such sources as erosion, placer mining, mine tailing wastes, strip mining, gravel washing, dusts from coal washeries, loose soils from freshly plowed farm lands, highway, and building projects The organic fraction includes such settleable materials as greases, oils, tars, animal and vegetable fats, paper mill fibers, synthetic plastic fibers, sawdust, hair, greases from tanneries, and various settleable materials from city sewers These solids may settle out rapidly and bottom deposits are often a mixture of both inorganic and organic solids They may adversely affect fisheries by covering the bottom of the stream or lake with a blanket of material that destroys the bottom fauna or the spawning grounds of fish Deposits containing organic materials may deplete bottom oxygen supplies and produce hydrogen sulfide, carbon dioxide, methane, or other noxious gases

Some settleable solids may cause damage by mechanical action

Water Quality Criteria for European Freshwater Fish (European Inland Fisheries Advisory Commission, 1964) discusses chemically inert solids in waters that are otherwise satisfactory for the maintenance of freshwater fisheries. It is indicated that good or moderate fisheries can be maintained in waters that normally contain 25 to 80 mg/l suspended solids, but that the yield of fish might be lower than in waters containing 25 mg/l or less Waters normally containing 80 to 400 mg/l suspended solids are unlikely to support good freshwater fisheries

Recommendation: Since it is known that even minor deposits of settleable materials inhibit the growth of normal stream or lake flora and fauna, it is recommended that no settleable materials be added to these waters in quantities that adversely affect the natural biota

Color

The color of water is attributed to substances in solution after the suspensoids have been removed It may be of organic or mineral origin Organic sources are humic materials, peat, plankton, rooted and floating aquatic plants, tannins, etc Inorganic sources are metallic substances such as iron and manganese compounds and chemicals, dyes, etc Many industries discharge materials that contribute to the color of water Among them are pulp and paper mills, textile mills, refineries, manufacturers of chemicals and dyes, explosives, nailworks, tanneries, etc

Standard Methods for the Examination of Water and Wastewater, 12th edition (1965), describes the standard platinum-cobalt method of determining color after centrifugation The unit of color considered as standard is the color produced by one mg/l of platinum in water Results are expressed as units of color. Color in excess of 50 units may limit photosynthesis and have a deleterious effect upon aquatic life, particularly phytoplankton, and the benthos

Water absorbs light differentially A layer of distilled water 1 meter in thickness absorbs 53 percent of the solar radiation It absorbs 30 percent of the red-orange band (6,500 angstrom units) but less than 5 percent of the blue (4,500 angstrom units). These are the portions of the spectrum that are absorbed and utilized to the greatest extent by chlorophyll The band at 7,500 angstrom units is over 90 percent absorbed

Natural waters absorb far more light The light intensity at which the amount of oxygen produced photosynthetically is balanced by the amount of oxygen used for respiration in some submerged vascular plants is 5% of full sunlight on clear summer days It is estimated that 25 to 50 percent of full sunlight is necessary for many green aquatic plants to reach maximum photosynthesis The ORSANCO committee observed that the 25-percent level of solar radiation is not reached in many of the larger streams and they considered it desirable to restrict the addition of any substances that reduce light penetration and hence limit the primary productivity of aquatic vegetation

Recommendation: For effective photosynthetic production of oxygen, it has been found that at least 10 percent of incident light is required. Therefore, 10 percent of the incident light should reach the bottom of any desired photosynthetic zone in which adequate dissolved oxygen levels are to be maintained

Floating materials

Floating materials include sawdust, peelings and other cannery wastes, hair and fatty materials from tanneries, wood fibers, containers, scums, oil, garbage, floating materials from untreated municipal and industrial wastes, tars and greases, and precipitated chemicals

Wastes from paper mills, vinegar plants, cane mills, and other industries may contribute nutrients or produce conditions in streams that foster the growth of *Sphaerotilus* (Chlamydobacteriales)[1] or similar iron or sulfur bacteria. These floating growths not only clog fishermen's nets, but also smother out the spawning grounds and habitat of all forms of aquatic life

Recommendation: All such floating and settleable substances should be excluded from streams and lakes

Tainting substances

Among the materials that are responsible for objectionable tastes in fish are hydrocarbons, phenolic compounds, sodium pentachlorophenate (used for slime control in cooling towers), coal tar wastes, gas wastes, sewage containing phenols, coal-coking wastes, outboard motor exhaust wastes, and petroleum refinery wastes Kraft paper mill wastes, sulfides, mercaptans, turpentine, wastes from synthetic rubber and explosives factories, algae, resins and resin acids also contribute to objectional tastes in fish Twenty gallons per acre of kerosene or diesel fuel will produce an off-flavor in bass and bluegills which persists for 4 to 6 weeks The Aquatic Life Advisory Committee of ORSANCO in its Third Progress Report (1960), lists the concentrations (table III–3) of phenolic substances that cause taste and odor Albersmeyer and Erichsen (1959) found that carbolated oil and light oil, both dephenolated, impart a taste to fish flesh more pronounced than that caused by naphthalene and methyl naphthalene They concluded that the hydrocarbons are more responsible for tastes in fish flesh than the phenolic compounds. Boetius (1954) found that chlorophenol could produce unpleasant flavor in fish at a concentration of only 0 0001 mg/l.

TABLE III–3. Concentration of Phenolic Compounds That Cause Tainting of Fish Flesh

After Bandt (1955) page 77 (except for phenol)

Compound or waste	Concentration affecting taste and odor (mg/l)	Fish tested
Pure compounds:		
Phenol	15 to 25	Trout, carp, tench, chub, eel, minnow, perch, bluegill, pike, goldfish
Cresols	10	Tench, carp, eel, trout, minnow
Xylenols	1 to 5	Roach, perch, carp
Pyrocatechol	2 to 5	Perch, carp, roach.
Pyrogallol	20 to 30	Roach, carp
P-Quinone	0 5	Carp, tench, roach
Pyridine	5	Roach, carp
Naphthalene	1	Roach
Alpha Naphthol	0 5	Roach, carp.
Quinoline	0 5 to 1 0	do
Chlorophenol	0 1	
Mixed phenolic wastes:		
Coal-coking wastes	0 02 to 0 1	Freshwater fish.
Coal-tar wastes	0 1	do
Phenols in polluted river	0 02 to 0 15	Minnows
Sewage containing phenols	0 1	Freshwater fish.

A preliminary laboratory study (English, McDermott, and Henderson, 1963) shows that outboard motor exhaust damages the quality of water in several ways, the most noticeable of which is causing unpleasant taste and odor in the water and off-flavoring of fish flesh. A later field study, English et al. (1963a, b) and Surber et al. (1965) determined the threshold level of tainting of fish in pond and lake waters to be about 2.6 gal/acre-foot of fuel, accumulating over a 2-month period. The gasoline used was regular grade and the lubricating oil (½ pint/gal) was a popular brand of packaged outboard motor oil

Recommendation: Materials that impart odor or taste to fish flesh or other freshwater edible products such as crayfish, clams, prawns, etc, should not be allowed to enter receiving waters at levels that produce tainting Where it seems probable that a discharge may result in tainting of edible aquatic products, bioassays and taste panels are suggested for determining whether tainting is likely

Radioactive materials in fresh and marine waters

Ionizing radiation, when absorbed in living tissue in quantities substantially above that of natural background, is recognized as injurious It is necessary, therefore, to prevent excessive levels of radiation from reaching any organism we wish to preserve, be it human, fish, or invertebrate. Beyond the obvious fact that they emit ionizing radiation, radioactive wastes are similar in many respects to other chemical wastes. Man's senses cannot detect radiation unless it is present in massive amounts Radiation can be detected, however, by means of electronic instruments and quantities present at very low levels in the environment can be measured with remarkable accuracy. Because of the potential danger, the disposal of radioactive materials has been well planned and controlled Injuries and loss of life from disposal of radioactive materials or from accidents involving these materials have been minimal Four factors have contributed to this safety record. (1) scientists and legislators were aware of the dangers associated with the release of radioactive materials into the environment prior to the need for disposal; (2) research has progressed to protect man against radiation effects and levels of radiation that could be released, (3) as knowledge of nuclear energy increased, standards were developed for handling, shipping, and disposing of radioactive substances; and (4) an extensive monitoring program was inaugurated and has been functioning for years.

Upon introduction into an aquatic environment, radioactive wastes can. (1) remain in solution or in suspension, (2) precipitate and settle to the bottom, or (3) be taken up by plants and animals. Immediately upon introduction of radioactive materials into the water, certain factors interact to dilute and disperse these materials, while simultaneously other factors tend to concentrate the radioactivity Among those factors that dilute and disperse radioactivity are currents, turbulent diffusion, isotopic dilution, and biological transport Radioactivity is concentrated biologically by uptake directly from the water and passage through food webs, chemically and physically by adsorption, ion exchange, coprecipitation, flocculation, and sedimentation

Radioactive wastes in the aquatic environment may be cycled through water, sediment, and the biota. Each radionuclide tends to take a characteristic route and has its own rate of movement from component to component prior to coming to rest in a temporary reservoir, one of the three components of the ecosystem Isotopes can move from the water to the sediments or to the biota. In effect, the sediments and biota compete for the isotopes in the water Even though in some instances sediments are initially successful in removing large quantities of radionuclides from the water, and thus preventing their immediate uptake by the biota, this sediment-associated radioactivity may later affect many benthic species by exposing them to radiation Also, any radioactivity leached from the sediments back to the water again becomes available for uptake by the biota Even before the radioactivity is leached from the sediment, it may become available to the biota due to a variation in the strength of the bonds between the different radionuclides and the sediment particles Loosely bound radionuclides can be "stripped" from particles of sediment and utilized by bottom-feeding organisms.

Plants and animals, to be of any significance in the cycling of radionuclides in the aquatic environment, must accumulate the radionuclide, retain it, be eaten by another organism, and be digestible However, even if an organism accumulates and retains a radionuclide and is not eaten before it dies, the radionuclide will enter the "biological cycle" through organisms that decompose the dead organic material into its elemental components Plants and animals that become radioactive in this biological cycle can pose a health hazard when eaten by man

Aquatic life may receive radiation from radionuclides present in the water and substrate and also from radionuclides that may accumulate within their tissues Humans can acquire radionuclides via many pathways, but among the most important are drinking water or edible fish and shellfish that have concentrated nuclides from the water In order to prevent unacceptable doses of radiation from reaching humans, fish, and other important organisms, the concentrations of radionuclides in water, both fresh and marine, must be restricted

The effects of radiation on organisms have been the subject of intense investigation for many years Careful consideration of pertinent portions of the vast amount of available information by such organizations as the International Commission on Radiological Protection (ICRP), the National Committee on Radiation Protection and Measurements (NCRP), and the Federal Radiation Council (FRC) has resulted in recommendations on the maximum doses of radiation that people may be allowed to receive under various circumstances (U S. Department of Commerce, 1963) The recommended levels for the general public are substantially more conservative than those for persons who work with radiation sources or radionuclides, but in both cases the recommended levels assume that the exposure will be sustained essentially throughout the life or period of employment of the person

The ICRP and NCRP have calculated the quantities of individual radionuclides that a person can ingest each day without accumulating levels in various body organs that deliver radiation doses in excess of the recommended limits These quantities contained in the volume of water ingested daily (2 2 liters) are referred to as "maximum permissible concentrations (MPC) in water" The FRC, recognizing that people may ingest radionuclides from foods and other sources as well as from drinking water, has provided guidance on the basis of transient rates of intake from all sources, but only for a few nuclides (radium-226, iodine-131, strontium-90, and strontium-89).

The PHS Drinking Water Standards (USDHEW, 1962) are responsive to the recommendations of the FRC, ICRP, and NCRP, and provide appropriate protection against unacceptable radiation dose levels to people where drinking water is the only significant source of exposure above natural background. Where fish or other fresh or marine products that have accumulated radioactive materials are used as food by humans, the concentrations of the nuclides in the water must be further restricted to provide assurance that the total intake of radionuclides from all sources will not exceed the recommended levels.

The radiation dose received by fish and other aquatic forms will be greater than that received by people who drink the water or eat the fish Even so, this does not place the fish in risk of suffering radiation damage The radiation protection guides for people have been established with prudence, for continued exposure over a normal life span, and with appropriate risk (safety) factors Virtually all of the available evidence shows that the concentrations of radionuclides in fish and shellfish that would limit their use as food are substantially below the concentrations that would injure the organisms from radiation Therefore, at this time there appears to be no need for establishing separate criteria for radioactive materials in water beyond those needed to limit the intake to humans.

Recommendation. (1) No radioactive materials should be present in receiving waters as a consequence of the failure of an installation to exercise practical and economical controls to minimize releases This recommendation is responsive to the recommendations of the FRC that "There can be no single permissible or acceptable level of exposure without regard to the reason for permitting the exposure It should be general practice to reduce exposure to radiation, and positive effort should be carried out to fulfill the sense of these recommendations It is basic that exposure to radiation should result from a real determination of its necessity"

(2) No radionuclide or mixture of radionuclides should be present at concentrations greater than those specified in the PHS Drinking Water standards (USDHEW, 1962) This recommendation assures that people will receive no more than acceptable amounts

of radioactive materials from aquatic sources and that fish living in the water will not receive an injurious dose of radiation.

(3) The concentrations of radioactive materials present in fresh, estuarine, and marine waters should be less than those that would require restrictions on the use of organisms harvested from the area in order to meet the Radiation Protection Guides recommended by the Federal Radiation Council

This recommendation assures that fish and other fresh water and marine organisms will not accumulate radionuclides to levels that would make them unacceptable for human food. It also limits the radiation dose that the organisms would receive from internally deposited nuclides to levels below those that may be injurious Some workers (Carritt, 1959; Isaacs, 1962; Pritchard, 1959) have recommended "maximum permissible levels for sea water" based on various assumptions of dispersion, uptake by marine organisms, and the use of the organisms as food by people While these recommendations are most useful as a first approximation in predicting safe rates of discharge of radioactive wastes, their applicability as water quality criteria is limited and they are not intended for use in fresh or estuarine waters where the concentrations of a great variety of chemical elements vary widely. Because it is not practical to generalize on the extent to which many of the important radionuclides will be concentrated by fresh water and marine forms, nor on the extent to which these organisms will be used for food by people, no attempt is made here to specify MPC for either sea water or fresh water in reference to uptake by the organisms Rather, each case requires a separate evaluation that takes into account the peculiar features of the region. Such an evaluation should be approved by an agency of the State or Federal Government in each instance of radioactive contamination in the environment. In each particular instance of contamination, the organisms present, the extent to which these organisms concentrate the radionuclides, and the extent to which man uses the organisms as food must be determined, as well as the rates of release of radionuclides must be based on this information

Plant nutrients and nuisance organisms

All terrestrial biological processes plus the majority of man's activities ultimately result in waste products in various stages of decomposition A portion of these sooner or later enter surface freshwaters These waste products include a rather abundant amount of plant nutrients such as nitrogen, phosphorus, carbon, and other elements Subsequently, these plant nutrients are incorporated into organic matter by aquatic plants.

Surface water areas are like land areas in that some type of vegetation will occupy any suitable habitat. Thus, the more abundant the nutrient supply, the more dense the vegetation, provided other environmental factors are favorable. In the aquatic habitat, these growths may be bacteria, aquatic fungi, phytoplankton, filamentous algae, submersed, emersed, floating, and marginal water plants. Practically all aquatic plants may be desirable at one time or another and in one habitat or another. However, when they become too dense or interfere with other uses of the water or of the aquatic habitat, they become nuisance growths.

Some sheath-forming bacteria are the primary nuisance-type growths in rivers, lakes, and ponds. A notable problem associated with this group occurs in areas subjected to organic enrichment. The most common offenders belong to the genus *Sphaerotilus* These bacteria are prevalent in areas receiving raw domestic sewage, improperly stabilized paper pulp effluents or effluents containing simple sugars. The growths they produce interfere with fishing by fouling lines, clogging nets, and generally creating unsightly conditions in the infested area Their metabolic demands while they are living and their decomposition after death impose a high BOD load on the stream and can severely deplete the dissolved oxygen. It has been suggested that large populations of *Sphaerotilus* render the habitat noxious to animals and hence its presence may actively exclude desirable fish and invertebrates

The freshwater algae are diverse in shape, color, size, and habitat A description of all species of algae would be as comprehensive as writing about all land plants, mosses, ferns, fungi, and seed plants

They may be free floating (planktonic) or they may grow attached to the substrate (benthic or epiphytic types). They may be macroscopic or microscopic and are single-celled, colonial, or filamentous They are the basic link in the conversion of inorganic constituents in water into organic matter When present in sufficient numbers, these plants impart a green, yellow, red, or black color to the water They may also congregate at or near the water surface and form so-called "waterbloom" or "scum."

A major beneficial role of algae is the removal of carbon dioxide from the water by photosynthesis during daylight and the production of oxygen Algae, like other organisms, continually respire and produce carbon dioxide The amount of oxygen produced during active photosynthesis is many times the amount of carbon dioxide released during the night or on cloudy days when photosynthesis is inhibited or stopped.

Limited concentrations of algae are not troublesome in surface waters; however, overproduction of various species is considered undesirable for many water uses A relatively abundant growth of planktonic algae in waters 3 feet or deeper will shade the bottom muds sufficiently to prevent germination of seeds and halt the growth of practically all rooted submersed and emersed aquatics, thus removing an important source of food for ducks and other water fowl

Some blue-green algae, many green algae, and some diatoms produce odors and scums that make waters less desirable for swimming Dense growths of such planktonic algae may limit photosynthetic activity to a layer only a few inches beneath the surface of the water. Under certain conditions, the populations of algae may die and their decomposition will deplete dissolved oxygen in the entire body of water. Certain sensitive people are allergic to many species of planktonic algae blooming in waters used for swimming

It is claimed that some species of algae cause gastric disturbances in humans who consume such infested waters Several species of blue-green algae produce, under certain conditions, toxic organic substances that kill fish, birds, and domestic animals Some of the genera that contain species which may produce toxins are *Anabaena, Anacystis, Aphanizomenon, Coleolosphaerium, Gloeotrichia, Microcystis, Nodularia,* and *Nostoc* Some species of *Chlorella,* a green alga, also are toxic

Various species of single, as well as branched filamentous forms of algae, grow in both cool and warm weather and when they become overabundant are generally considered to be a nuisance in whatever body of water they occur. Most species of these algae are generally distributed over the United States

Many forms of plankton and filamentous algae clog sand filters in water treatment plants, produce undesirable tastes and odors in drinking water, and secrete oily substances that interfere with domestic use and manufacturing processes Some algae cause water to foam during heating as well as metal corrosion and the clogging of screens, filters, and piping Algae also coat cooling towers and condensers causing these units to become ineffective. In Lake Superior, complaints have been made that diatoms such as *Tabellaria, Synedra, Cymbella,* and *Fragilaria,* and the chrysophyte, *Dinobryon,* may be the cause of slimes on fishnets

Filamentous algae may interfere with the operation of irrigation systems by clogging ditches, wires, and screens and thus seriously impede the flow of water. Filamentous algae in ponds, lakes, and reservoirs may cause depletion of naturally occurring and added nutrients that could otherwise be used to produce unicellular algae that are more commonly used as food by fish Dense growths of filamentous algae may reduce the total fish production and seriously interfere with harvesting the fish either by hook and line fishing, seining, or draining. Such growths can also cause overpopulation, resulting in stunting and the presence of large numbers of small fish Under certain conditions, growths of filamentous algae on pond or lake bottoms become so dense that they eliminate spawning areas of fish and possibly interfere with the production of invertebrate fish food.

Submersed plants are those which produce all or most of their vegetative growth beneath the water surface. In many instances these plants have an underwater leaf form, a totally different floating or emersed leaf form, and flowers on an aerial stalk Abundant growth of these weeds is dependent upon depth and turbidity of water, and substratum For most submersed plants in clear water, 8 to 10 feet is the maximum depth for growth in clear water as they must receive sufficient light for photosynthesis when they are seedlings Most of these submersed aquatic plants appear capable of absorbing nutrients as well as herbicides through either their roots or vegetative parts

Emersed plants are rooted in bottom muds and produce a majority of their leaves and flowers at or above the water surface Some species have leaves that are flat and float entirely upon the water surface Other species have leaves that are saucer-shaped or whose margins are irregular or fluted The latter types of leaves do not float entirely upon the water surface

Marginal plants are probably the most widely distributed of the rooted aquatic plants Members of this group are varied in size, shape, and preference of habitat Many species are adapted for growth from moist soils into water up to 2 feet deep or more Other species are limited to moist soil or entirely to a watery habitat

There are some species of floating plants that are rather limited in their distribution while others are widespread throughout the world Plants in this group have true roots and leaves, but instead of being anchored in the soil they float about on the water surface Buoyancy of the plant is accomplished through modification of the leaf (including covering of the leaf surface) and leaf petiole Most species have well-developed root systems which collect nutrients from the water.

Species designated as weeds are not necessarily such in all places and at all times. For example, many submersed, floating, and emersed plants that normally interfere with boating, swimming, and fishing are regarded as desirable growths in waterfowl refuge areas Rooted plants with floating leaves, such as water lillies and watershield, and those that float upon the surface, such as water hyacinth, elodea, parrotweed, alligatorweed, and duckweed, are considered highly objectionable for many water uses. In clear water areas, however, where artificial or natural fertilization is moderate, the removal of these surface-shading plants may permit sunlight to penetrate to the bottom muds and submersed plants soon will occupy these waters These submersed plants generally are more objectionable in an area than the original surface-covering plants.

Most emersed, marginal, and a few submersed plants and filamentous algae produce growths that provide a suitable habitat for the development of anopheline and other pest-type mosquitoes as well as a hiding place for snakes. They are excellent habitats for damselflies and some aquatic beetles

Most rooted and floating aquatic plants can seriously interfere with navigation of small recreational craft and large commercial boats in infested areas. Such problems are prevalent in intercoastal waterways and in some streams in the Gulf States area Water shortages due to consumption by undesirable aquatic plants or reduction in carrying capacity of an irrigation or drainage canal through excessive vegetation can result in decreased crop quality, yield, or even crop failure.

Submersed and emersed weeds consume nutrients, either available or added, that could otherwise be used to grow desirable planktonic algae in impounded waters Thus, the presence of excessive rooted plants may reduce total fish production in the infested body of water Extensive growth of weeds provides dense cover that allows the survival of excessive numbers of fish resulting in overcrowding and stunting as well as interfering with harvesting the fish by hook and line or other methods There is evidence that rank growths of submersed, emersed, or floating weeds may deplete the dissolved oxygen supply in shallower water and that fish tend to leave these areas if there are open-water areas available of better quality Although they carry on the process of photosynthesis, their multicellular structure often makes them less effective in re-oxygenating the water

All the elements essential for plant growth are yet to be determined Some of the elements known to be important are nitrogen, phosphorus, potassium, magnesium, calcium, manganese, iron, silicon (for diatoms), sulfur (as sulfates), oxygen, and carbon. In many habitats, abundance of the first two elements, N (nitrogen) and P (phosphorus), promotes vegetative production if other conditions for growth are favorable. Most algae also require some simple organics, such as amino acids and vitamins, and many trace elements, such as manganese and copper Not only are the various factors important, but their relative abundance and combined affect can be of even greater importance. Limited laboratory studies made to date indicate that different species of algae have somewhat different phosphorus requirements with the range of available phosphorus usually falling between 0 01 and 0 05 mg/l as phosphorus. At these levels, when other conditions are favorable, blooms may be expected. As has been pointed out by the Subcommittee on Water Quality Criteria for Public Water Supply, the total phosphorus is of outstanding importance While there is no set relationship between total and available phosphorus (because the ratio varies with season, temperature, and plant growth), the total phosphorus is governing as it is the reservoir that supplies the available phosphorus It is believed that allowable total phosphorus depends upon a variety of factors; e g, type of water, character of bottom soil, turbidity, temperature, and especially desired water use. Allowable amounts of total phosphorus will vary, but in general it is believed that a desirable guideline is 100 μg/l for rivers and 50 μg/l where streams enter lakes or reservoirs (recommended by the Public Water Supply Subcommittee)

The nitrogen-phosphorus ratio is also of importance The ratio varies with the water, season, temperature, and geological formation, and may range from 1 or 2 1 to 100 1 In natural waters, the ratio is often very near 10 1, and this appears to be a good guideline for indicating normal conditions

The major sources of nitrogen entering fresh waters are atmospheric (approximately 5 lbs/acre/year), (Hutchinsen, 1957), domestic sewage effluents, animal and plant processing wastes, animal manure, fertilizer and chemical manufacturing spillage, various types of industrial effluents, and agricultural runoff

The major sources of phosphorous entering fresh waters are domestic sewage effluents (including detergents), animal and plant processing wastes, fertilizer and chemical manufacturing spillage, various industrial effluents, and, to a limited extent, erosion materials in agricultural runoff.

Phosphorous entering an ecosystem may produce a high oxygen demand. It has been pointed out that 1 milligram of phosphorous from an organic source demands about 160 milligrams of oxygen in a single pass through the phosphorus cycle to complete oxidation. Thus the oxidation of organic matter, the growth of which has been induced by adding phosphorus, may bring about a great reduction of oxygen in a lake or stream

Dissolved carbon in the form of simple organic compounds can be utilized by many kinds of algae. These types of carbon compounds are also used directly as a source of food by many animals Varying amounts of simple organic compounds containing carbon are found in sewage and several types of industrial wastes. Other more complex forms of organic carbon can be utilized by bacteria The most common nuisance growth that becomes very abundant in the presence of very small amounts of carbon is *Sphaerotilus*. Patrick (unpublished data) has shown that the addition of 0 05–0.1 mg/l of glucose, without changing other ecological conditions, may produce nuisance growths.

Knowledge of the nutrient requirements of fungi, phytoplankton, and filamentous algae is more extensive than for rooted aquatic plants Laboratory data on nutrient requirements must be used with caution, however, because the maintenance of most long-term cultures has required that extracts of soil be incorporated into the inorganic culture medium Analyses of field grown algae have indicated a wide divergence in elemental composition among various species and among the same species from different localities Excessive growths often seen to be triggered by small amounts of so-called minor or trace elements and vitamins, particularly B_{12}

One of the most obvious effects of increases or imbalances in nutrients is the change in the kinds and abundance of species composing the algal flora Historical studies of Lake Erie show a change from an *Asterionella* dominance in the spring and a *Synedra* dominance in the fall of 1920 to a *Melosira* dominance in the spring and a *Melosira, Anabaena, Oscillatoria* dominance in the fall of 1962. Between 1919 and 1934, the number of cells per ml, with two exceptions, always were less than 4,000/ml Since 1934, the cell count, with one exception, has always been greater than 4,000/ml. In 1944, it reached 11,032 cells/ml. It should be pointed out that blue-green algae are a poor source of food for most aquatic life.

Benthic forms also indicate the increase in nutrients in an ecosystem Various species of *Cladophora* become abundant in lakes and rivers when nutrients are abundant and replace the original diverse benthic flora.

This demand for a wide variety of nutrients is also characteristic of many of the rooted aquatic plants. Their affinity for numerous metals, however, does not appear to be comparable to that of the algae

Extensive data exist on the concentration of nitrogen and phosphorus in fresh waters throughout the United States. (Allee, et al., 1949; Ellis, 1940; Engelbrecht and Morgan, 1961; Juday, et al , 1927; Lackey, 1945; USDHEW, 1962a) In evaluating these data, it must be remembered that algae and most other aquatic plants are capable of utilizing any available N and/or P in a very short time providing other growth conditions are favorable. Thus, analyses of filtered water would not provide an evaluation of all elements existing in the original water sample. A more meaningful figure would result if all materials in an original water sample were digested and then analyzed. Often, the dissolved or available phosphorus may be very low, while the total amount in the organisms and organic matter may be quite large. Not only does this determination of total phosphorous give a better estimate of the existing nutrient load of an area, but it also provides an index to the potential release that would occur if these plants should all die within a short period of time

This information would also point out the fact that in many freshwaters, various species of rooted aquatic plants are excellent receptors for this nutrient load Their use in effluent treatment might be one of the cheaper waste-treatment procedures The chemical composition of several species of plants is given in table III–4. Indications are that the N–P content of freshwaters in the United States is quite varied, and their presence in fairly large amounts may or may not produce algae blooms.

It must be remembered that factors other than plant nutrients also are operative in the establishment and maintenance of aquatic plant growths. There must be sufficient light reaching the plant for photosynthesis to occur. If turbidity from muds, dyes, other materials, or even phytoplankton is too great, plants at lower depths cannot grow These same plants, however, if established in an area, can trap large amounts of intermittent silt and other materials and clear the waters for downstream uses

Another factor that might be operative in preventing aquatic plant growth would be the lack of free CO_2 and bicarbonate ions in a particular aquatic environment. Certainly in an area where the pH is high (9.5 or above) or low (below 5 5), productivity would not reach high levels due to a lack of sufficient bicarbonates.

Temperature also is an important factor in determining the amount of growth. For each species, there is an optimum range in which the greatest growth occurs.

Wave action on large expanses of water may also be a factor in regulating all types of aquatic plant growths. This appears contradictory to the concept that winds cause mixing of surface and bottom waters, thereby renewing plant nutrients in the euphotic zone. However, in certain lakes and reservoirs, wind-induced waves and currents mechanically agitate bottom materials and waters to an extent that interferes with the production of phytoplankton and rooted aquatic plants

Various workers have discussed the concentrations of nitrogen and phosphorus that are needed for an algal bloom. Sawyer (1947) suggests that a concentration of at least 15 μg/l of phosphorus is necessary for growth. Hutchinson (1957) states that *Asterionella* can take up phosphorus from where it is present at less than one μg/l. As a result of the study of 17 Wisconsin lakes, Mackenthun (1965) cites results indicating that inorganic nitrogen at 0.30 mg/l and inorganic phosphorus at 0.01 mg/l, at the start of an active growing season, subsequently permitted algal blooms. As yet, there is no definite information on the amount of wastes that will produce predictable harmful effects in a lake There are indicators, however, of developing or potentially undesirable conditions

There are several conditions, analyses, or measures that will indicate eutrophication and dystrophication Since these parameters are not infallible, it is well to use them in combinations Conditions indicative of organic enrichment are:

(1) A slow overall decrease year after year in the dissolved oxygen in the hypolimnion as indicated by determinations made a short time before the fall overturn and an increase in anaerobic areas in the lower portion of the hypolimnion

TABLE III–4. Chemical Composition of Some Algae From Ponds and Lakes in Southeastern United States [1]

Analysis	Chara	Pithophora	Spirogyra	Giant Spirogyra	Rhizoclonium	Oedogonium	Mougeotis	Anabaena
Ash percent	43 4	27 77	13 06	13 86	17 36	12 69	14 54	5.19
C percent	29 3	35 38	42 40	41 16	39.10	40 84	40 74	49 70
N percent	2 46	2 57	3 01	2 35	3 46	2 64	1 77	9 43
P percent	0 25	0 30	0 20	0 23	0 43	0 08	0 25	0 77
S percent	0 55	1 42	0 27	0 24	0 27	0 15	0 36	0 53
Ca percent	8 03	3 82	0 57	0 84	0 52	0 44	1 68	0 36
Mg percent	0 92	0 20	0 45	0.30	0 21	0 16	0 57	0 42
K percent	2 35	3 06	0 92	0 99	1 90	3 03	1 20	1 20
Na percent	0 13	0 07	1 42	1 43	0 09	0 06	0 49	0 18
Fe mg/l	2,520	2,836	1,368	1,793	1,820	1,645	60	80
Mn mg/l	2,926	829	1,641	1,658	1,687	1,729	1,080	800
Zn mg/l	89	29	72	46	89	119	520	---
Cu mg/l	19	23	47	34	75	75	143	70
B mg/l	6 7	65	4 2	4 3	1 8	8 1	8	---

Analysis	Cladophora	Euglena	Hydrodictyon	Microcystis	Lyngbya	Nitella	Aphanizomenon
Ash percent	23 38	4 12	17 94	6 2	17 20	19 11	7 21
C percent	35 27	48 14	39 96	46 46	40 23	38 43	47.65
N percent	2 30	5 14	3 87	8 08	5 01	2 70	8 57
P percent	0 56	0 67	0 24	0 68	0 31	0 23	1 17
S percent	1 58	0 19	1 41	0 27	0 28	0 34	1 18
Ca percent	1 69	0.05	0 69	0 53	0 45	1 89	0 73
Mg percent	0 23	0 07	0 17	0 17	0 14	0 95	0 21
K percent	6 08	0 34	4 21	0 79	0 42	3.73	0.68
Na percent	0 18	0 02	0 38	0 04	0 06	0 28	0.19
Mn mg/l	1,040	240	1,373	2,751	5,230	2,388	167
Fe mg/l	2,300	1,545	1,963	322	3,866	2,180	833
Zn mg/l	10	73	129	48	171	240	120
Cu mg/l	190	290	114	37	101	39	187
B mg/l	84 6	3 8	---	3 6	112	9 8	---

[1] Lawrence (personal communication)

(2) An increase in dissolved solids—especially nutrient materials such as nitrogen, phosphorus, and simple carbohydrates.

(3) An increase in suspended solids—especially organic materials.

(4) A shift from a diatom-dominated plankton population to one dominated by blue-green and/or green algae, associated with increases in amounts and changes in relative abundance of nutrients

(5) A steady though slow decrease in light penetration.

(6) An increase in organic materials and nutrients, especially phosphorus, in bottom deposits.

Recommendation: The Subcommittee wishes to stress that the concentrations set forth are suggested solely as guidelines and the maintenance of these may or may not prevent undesirable blooms All the factors causing nuisance plant growths and the level of each which should not be exceeded are not known

(1) In order to limit nuisance growths, the addition of all organic wastes such as sewage, food processing, cannery, and industrial wastes containing nutrients, vitamins, trace elements, and growth stimulants should be carefully controlled. Furthermore, it should be pointed out that the addition of sulfates or manganese oxide to a lake should be limited if iron is present in the hypolimnion as they may increase the quantity of available phosphorus

(2) Nothing should be added that causes an increased zone of anaerobic decomposition of a lake or reservoir

(3) The naturally occurring ratios and amounts of nitrogen (particularly NO_3 and NH_4) to total phosphorus should not be radically changed by the addition of materials As a guideline, the concentration of total phosphorus should not be increased to levels exceeding 100 μg/l in flowing streams or 50 μg/l where streams enter lakes or reservoirs

(4) Because of our present limited knowledge of conditions promoting nuisance growth, we must have a biological monitoring program to determine the effectiveness of the control measure put into operation A monitoring program can detect in their early stages the development of undesirable changes in amounts and kinds of rooted aquatics and the condition of algal growths With periodic monitoring such undesirable trends can be detected and corrected by more stringent regulation of added organics

Toxic substances

Aquatic life too frequently is considered only in terms of harvestable species. The fact that numerous other organisms are essential to produce a crop of fishes often is overlooked or given little attention. To produce a harvestable crop of fish, it is essential to have supporting plants and animals for food Requirements are established on the basis that the needed criteria are those that will protect fish, the harvested crop, and the food organisms necessary to support that crop. At this time, it is believed that every important species should be protected. One can appreciate that unimportant organisms may be sacrificed if the following criteria are adopted. Fish too often are considered as a single species instead of a multitude of species. Many are distinctly and greatly different from other related species and have their own distinctive requirements. Because of this, and because the important species, *essential* food organisms, and water quality will be different in different habitats, a single value or concentration has very limited applicability unless appropriate margins of safety are incorporated.

For these reasons, the bioassay approach described later in this section is favored It is believed *that bioassays are the best method for determining* safe concentrations of toxicants for the species of local importance Bioassays are essential also to determine safe concentrations for food organisms of those species and the effect of existing water quality, including environmental variables as well as existing pollution. Pertinent to this stance is the fact that the majority of specific pollution problems are ones involving discharges of unknown and variable composition. Almost without exception, more than one toxicant or stress is present Further, suggested safe concentrations probably will not be adequate in instances where more than one adverse factor exists. It is believed that these recommended levels will be adequate for a particular pollutant if dissolved oxygen, temperature, and pH are within the limits recommended If the latter parameters are outside recommended limits, appropriate alterations in the criteria for toxicants must be made.

Most of the available toxicity data are reported as the median tolerance limit (TL_m), the concentration that kills 50 percent of the test organisms within a specified time span, usually in 96 hours or less This system of reporting has been misapplied by some who have erroneously inferred that a TL_m value is a safe value, whereas it is merely the level at which half the test organisms are killed In many cases, the differences are great between TL_m concentrations and concentrations that are low enough to permit reproduction and growth

Substantial data on long-term effects and safe levels are available for only a few toxicants, perhaps 10 The effect of toxicants on reproduction is nearly unknown, yet this is a very important aspect of all long-term toxicity tests. In chronic tests with six different toxicants, there were three toxicants with which certain concentrations permitted indefinite survival and normal appearance but blocked spawning completely. Such evidence makes estimates of safe concentrations based on acute lethal test data alone very difficult and frequently erroneous. Equally problematical is the near-total lack of information on the sensitivity of the various life stages of organisms. Many organisms are the most sensitive in the larval, nymph, molting, or fry state; some may be the most sensitive in the egg and sperm stage

A further difficulty is encountered in recommending criteria because continuous acceptable concentrations must be lower than the intermittent concentrations that may be reached occasionally without causing damage. There seems to be only one way in which to resolve this difficulty and that is to use *both* maximum concentration and a range of concentrations. It is recognized that the extremes do limit organisms, but, within these extremes there is a range in concentration that can be tolerated and is safe for prolonged periods of exposure

Average 24-hour concentrations can be determined by using a small water pump to collect 1 to 5 ml samples every minute. After 24 hours, the sample is mixed and analyzed. The concentration found represents the average concentration. Samples obtained this way are more reproducible and easier to secure than the maximum instantaneous concentration. Maximum concentrations must be considered in the criteria, however, because an average concentration alone could be met and yet permit a lethal concentration to exist for a critical period.

Bioassay

The use of some type of bioassay to determine the toxicity of a material or waste can be the most effective and accurate method of assessing potential danger With these methods, no assumptions need be made concerning the chemical structure or form of the pollutant, nor does the investigator have to know the constituent substances The effects of water quality on toxicity also may be measured Naturally, the more that is known about the chemical and physical behavior of a toxicant in water, the more precise the assay can be.

While there are many types of assays, two are in general use: (1) the static bioassay in which the test solution is not changed during the period of exposure, and (2) the flow-through bioassay in which the test solution continually is renewed. It is nearly impossible in a static test to use the introduced test concentration for calculating TL_m values, especially for substances or wastes that are toxic at concentrations of 1 mg/l or less, because the quantity taken into the test organism may be a very large percentage of the amount contained in the test water. A 48-hour TL_m based on the introduced concentration could give a value much higher than the true concentration because of this decrease in toxicant concentration The initial test concentration is usually not measured in static tests because of the changing concentration. Knowledge of the concentration of the toxicant at the end of the test can be of value.

The static test can give useful relative measures of toxicity for wastes of high toxicity, but for the reason mentioned above, it should not be used for absolute values. Less toxic substances can be assayed much more accurately and lethal concentrations can be determined with confidence. The chemical nature of the tested substances has an important effect on the accuracy of the results as well. Substances that are volatile, unstable, or relatively insoluble may not be accurately assayed while substances having opposite properties can be assayed more accurately.

The problem of maintaining oxygen concentrations suitable for aquatic life in the test chamber can be very difficult Insufficient oxygen may be present in the test water volume because a BOD or COD may consume much of the available dissolved oxygen and aeration or oxygenation may degrade or remove the test material. Devices for maintaining satisfactory dissolved oxygen in static tests have been proposed and used with some degree of effectiveness A rather complete account of static assays can be found in Sandard Methods for the Examination of Water and Wastewater, 12th edition (1965), and Doudoroff, et al. (1951)

In the flow-through type of bioassay a device is used to add toxicant to a flow of water and the mixture is discharged into the test container. This method of testing has few of the problems mentioned in connection with the static test and has other advantages in addition. Its important disadvantage is the more complicated work of building the necessary equipment; namely, a water supply system, metering devices, and the provision of a large quantity of the test substance.

Its important advantages are that a predetermined concentration of test material can be maintained, oxygen concentrations can be kept high or be controlled, metabolites and waste products are removed (animals can be fed), absolute rather than relative TL_m values can be obtained, and volatile, unstable, and sparingly soluble materials can be tested. Additionally, multifactor experiments are possible in which several variables can be controlled (pH, dissolved oxygen, carbon dioxide, etc.). The constant renewal test is superior for monitoring effluents, water supplies, or streams on a continuous or intermittent basis and is the only suitable method for long-term tests.

Several systems for adding the test material to the water have been devised since this type of bioassay has been in use. Lemke and Mount (1963) describe a system using a controlled water flow balanced against a chemical metering pump Henderson and Pickering (1963) describe a simple drip system and a controlled water flow, a similar system is proposed by Jackson and Brungs (1966) Both of these latter references describe the use of fish and flowing systems as continuous monitors. Mount and Warner (1965), Mount and Brungs (1967), and Brungs and Mount (1967) describe systems suitable for continuous short or long-term tests

Most criteria for toxic substances must be based on a bioassay made for each specific situation This is dictated by the lack of information and the wide variation in situations, species, water quality, and the nature of the substance being added to the water.

Most of the bioassay work on algae has measured the threshold concentrations that reduce physiological processes by 50 percent rather than the concentrations that cause 50-percent death in the population tested. It is very difficult to determine the death point of algae cells, but some workers have used it as a criterion Physiological measurements have been based largely on 50-percent reduction in photosynthesis and 50-percent reduction in number of divisions that have taken place during a period of time. This is determined by the number of cells present at the beginning and end of the experiment A bioassay method employing diatoms has been recognized by the American Society for Testing Materials (1964)

Application Factors

Short-term or acute toxicity tests provide information on the overall toxicity of a material and thus precede meaningful long-term toxicity studies They may also be used to compare toxicities of different materials When water for dilution is taken from the receiving stream, these tests may also indicate additional stresses due to materials already present in the receiving water These acute studies do not indicate concentrations of a potential toxicant that are harmless under conditions of long-term exposure It is desirable, therefore, to develop a factor that can be used with 96 or 48-hour TL_m values to indicate concentrations of the waste or material in question that are safe in the receiving water Such a factor has been called an application factor

Ideally, an application factor should be determined for each waste or material. To do this, it would be necessary to determine the concentration of the waste or material in question that does not adversely affect the productivity of the aquatic biota on continuous exposure, in water of known quality, and under environmental conditions (DO, temperature, pH, etc) at which it is most toxic This concentration is then divided by the 96-hour TL_m value obtained under the same conditions to give the application factor.

$$\frac{\text{safe concentration for continuous exposure}}{\text{96-hour } TL_m}$$

For example, if the 96-hour TL_m is 0 5 mg/l and the concentration of the waste found to be safe is 0 01 mg/l, the application factor would be:

$$\frac{\text{safe concentration}}{\text{96-hour } TL_m} = \frac{0\ 01}{0\ 50} = \frac{1}{50}$$

In this instance, the application factor is $\frac{1}{50}$ or 0 02. Then in a given situation involving this waste, the safe concentration in the receiving stream would be found by multiplying the 96-hour TL_m by 0 02.

To effectively determine the application factor for a given waste, it is necessary to determine the concentration of that waste which is safe under a given set of conditions For those materials whose toxicity is not significantly influenced by water quality and in streams free of other wastes that influence the waste in question or that have water qualities similar to those under which the waste was tested, the above-mentioned concentration would be the one that is safe in the receiving water However, differences in water quality and lack of information on the toxicity of waste materials already present make the direct use of laboratory-determined safe levels unwise at present, and a different approach is recommended.

In this approach, a 96-hour TL_m is determined for the waste using water from the receiving stream for dilution and, as test organisms, the most sensitive species or life stage of an economically important local species or one whose relative sensitivity is known. This procedure would take into consideration the effects of local quality and the stress or adverse effects of wastes already present in the stream. The TL_m value thus found then is multiplied by the application factor for that waste to determine the safe concentration of that waste in that stream or stream section. Such bioassays should be repeated at least monthly and at each change in process or rate of waste discharge.

This procedure must be used because of the extreme difference in sensitivity among species and among necessary fish food organisms. Henderson (1957) has discussed various factors involved in developing application factors. Results of studies by Mount and Stephan (1967), in which continuous exposure was used, reveal that the application factor necessary to reduce the concentration low enough to permit spawning ranged from 1/7 to 1/200. It is recognized that exposure will not be constant in most cases and that higher concentrations usually can be tolerated for short periods.

At present, safe levels have been determined for only a few wastes and hence only a few application factors are known. Since the determination of safe levels is an involved process, it will be necessary to use indirect or stopgap procedures for estimating tolerable concentrations of various wastes in receiving waters To meet this situation, it is proposed to use three universal application factors selected on the basis of present knowledge, experience, and judgment. It is proposed that these general application factors be applied to TL_m values determined by those discharging wastes in the manner described above to set tolerable concentrations of their wastes in the receiving stream.

It should be evident that when these general application factors are used for all wastes the resulting concentrations at times will be more stringent than needed for some wastes and inadequate for others The derived concentrations will be tolerable, however, for a considerable number of wastes in the midrange of relative toxicity.

Recommendation for the Use of Bioassays and Application Factors To Denote Safe Concentrations of Wastes in Receiving Streams: (1) For the determination of acute toxicities, flow-through bioassays are the first choice. Methods for carrying out these flow-through tests have been described by Surber and Thatcher (1963), Lemke and Mount (1963), Henderson and Pickering (1963), Jackson and Brungs (1966), Mount and Warner (1965), Mount and Brungs (1967), and Brungs and Mount (1967) Flow-through bioassays should be used for unstable, volatile, or highly toxic wastes and those having an oxygen demand They also must be used when several variables such as pH, DO, CO_2 and other factors must be controlled

(2) When flow-through tests are not feasible, tests of a different type or duration must be used The kinds of local conditions affecting the procedure might be a single application of pesticides or lack of materials and equipment

(3) Acute static bioassays with fish for the determination of TL_m values should be carried out in accordance with Standard Methods for the Examination of Water and Wastewater, 12th edition (1965). Such tests should be used for the determination of TL_m values only for persistent, nonvolatile, or highly soluble materials of low toxicity which do not have an oxygen demand because it is necessary to consider the amount added as the concentration to which the test organisms are exposed

(4) When application factors are used with TL_m values to determine safe concentrations of a waste in a receiving water, the bioassay studies to determine TL_m values should be made with the most sensitive local species and life stages of economical or ecological importance and with dilution water taken from the receiving stream above the waste outfall Other species whose relative sensitivity is known can be used in the absence of knowledge concerning the most sensitive of the important local species or life stages or due to difficulty in providing them in sufficient numbers Alternatively, tests may be carried out using one species of diatom, one species of an invertebrate, and two species of fish, one of which should be a pan or game fish Further, these bioassays must be performed with environmental conditions at levels at which the waste is most toxic Tests should be repeated with one of the species at least monthly and when there are changes in the character or volume of the waste

(5) Concentration of materials that are nonpersistent (that is, have a half life of less than 96 hours) or have noncumulative effects after mixing with the receiving waters should not exceed 1/10 of the 96-hour TL_m value at any time or place. The 24-hour average of the concentration of these materials should not exceed 1/20 of the TL_m value after mixing. For other toxicants the concentrations should not exceed 1/20 and 1/100 of the TL_m value under the conditions described above. Where specific application factors have been determined, they will be used in all instances.

When two or more toxic materials whose effects are additive are present at the same time in the receiving water, some reduction in the permissible concentrations as derived from bioassays on individual substances or wastes is necessary The amount of reduction required is a function of both the number of toxic materials present and their concentrations in respect to the derived permissible concentration An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible concentration for the mixture is through the use of following relationship

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b}\qquad+\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b, C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b, L_n are the respective permissible concentration limits derived for the materials on an individual basis Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary

Heavy Metals

An extensive discussion of the physiological mode of action of heavy metals is found in the toxicity portion of the section on water quality requirements for marine organisms.

Zinc: While much information has been published regarding zinc, a large amount of the data cannot be used because of incomplete description of methods, type of water, or concentrations. The authors of many of the papers dealing with zinc toxicity have used various specific sublethal effects as endpoints and there is no way to compare these findings with other work

Since the concentration of calcium and magnesium influences heavy metal toxicity, permissible levels of heavy metals are dependent on the calcium and magnesium concentrations. Certain studies with zinc (Mount, 1966; and unpublished work of the FWPCA National Water Quality Lab., Duluth, Minn.) and cadmium indicate that for a given calcium and magnesium concentration the acute toxicity of zinc and cadmium increases (TL_m concentration decreases) as pH is raised from 5 to 9. This seems contrary to prevalent opinion that metal toxicity is related to metal in solution and that as pH increases (solubility decreases) the toxicity decreases. The reason for this apparent contradiction is that conceptions concerning the effect of pH are based on natural waters in which pH does not vary independently of calcium and magnesium concentrations, but rather is closely related to it. In those cases where this relationship has been studied, except for one (Sprague, 1964b), the toxicity has increased with an increase in pH. This concept also is consistent with the work of Lloyd (1961b) and, more recently, that of Herbert and Wakeford (1964) who concluded that colloidal or flocculated, but suspended, zinc exerts a toxic influence on fish.

The significance of temperature and the calcium-magnesium content on the toxicity of zinc to plankton has been pointed out by Patrick (unpublished data). In these tests, a 50-percent reduction in growth of the population was used as a measure. Results of these tests are summarized as follows:

Temperature of test solution	Concentration in mg/l which reduces growth of population by 50 percent	
	Ca-Mg concentration—44 mg/l as $CaCO_3$ Nitzchia linearis	Ca-Mg concentration—170 mg/l as $CaCO_3$ Navicula seminulum
72 F	4 29 mg/l	4 05 mg/l
82 F	1 59 mg/l	2 31 mg/l
86 F	1 32 mg/l	3.22 mg/l

Palmer (1957) found that zinc dimethyl dithiocarbamate (ZDD) inhibited growth of *Microcystis* at 0.004 mg/l A concentration of 0.25 mg/l controlled all diatoms, 43 percent of the blue-green algae, and 18 percent of the green algae. The above evidence implies that permissible levels of zinc cannot be related to the calcium-magnesium concentrations or to pH alone

Herbert and Wakeford (1964) described the effect of salinity on the toxicity of zinc to rainbow trout Since zinc was most toxic to trout in freshwater, it is assumed that concentrations which are safe in freshwater will be safe for the salmonids in brackish water. The maximum reported affect of a reduction of dissolved oxygen from 6–7 mg/l to 2 mg/l on the acute toxicity of zinc is a 50-percent increase in its acute toxicity (Lloyd, 1961a, Pickering, in press; Cairns and Scheier, 1958a). Since 4 mg/l is the minimum permitted, this effect is small in comparison to the difference between safe and acutely toxic concentrations. The use of an application factor, therefore, should provide adequate protection. Similarly, Herbert and Shurban (1963a) found that the 24-hour TL_m for zinc was reduced only 20 percent for rainbow trout forced to swim at 85 percent of their maximum sustained swimming speed

The effect of calcium and magnesium concentrations on the toxicity of zinc for plankton, invertebrates, fishes, and their embryonic stages is reflected in the spread of values reported as toxic by many sources (Anderson 1950, Brungs, in press; Cairns and Scheier 1957, 1958b; Grande, 1966, Herbert and Shurben, 1963a, b, Jones, 1938, Lloyd, 1961b, Patrick, personal communication; Pickering, in press, Pickering and Henderson, 1966a, Pickering and Vigor, 1965, Skidmore, 1964, Sprague, 1964a, b, Sprague and Ramsey, 1965; Williams and Mount, 1965; and Wurtz, 1962)

Recommendation: The relationship between calcium and magnesium concentration, pH, and zinc toxicity is confusing and the separate effects have been little studied Brungs (in press) has determined that 1/100 of the 96-hour TL_m value is a safe concentration for continuous exposure

Copper: The same general considerations apply to the determination of safe levels of copper as apply to safe levels for zinc and the discussion of copper will be based on the same assumptions. From the published data, differences in species sensitivity to copper appear to be somewhat greater than for zinc (Anderson, 1950; Grande, 1966; Herbert and Vandyke, 1964; Jones, 1938; Lloyd, 1961b, Mount, in press; Pickering and Henderson, 1966a; Sprague, 1964a, b; Sprague and Ramsey, 1965, Trama, 1954; Turnbull, DeMann, and Weston, 1954). Mount (in press) has found that 1/30 of the 96-hour TL_m value is a safe concentration for continuous exposure of fish.

Bringmann and Kuhn (1959a, b) report that 0 15 mg/l copper is the threshold concentration which produces a noticeable effect on *Scenedesmus*. Maloney and Palmer (1956) report that 0.5

mg/l copper as copper sulfate produces the following percents of death in algae:

57 percent in 17 species of blue-green algae
35 percent in 17 species of green algae
100 percent in 6 species of diatoms

Fitzgerald, Gerloff, and Borg (1952) report that 0 2 mg/l of copper (as copper sulfate) produces a 100-percent kill of *Microcystis aeruginosa*. Crance (1963) found 0.05 mg/l kills *Microcystis*. Hassall (1962), working with *Chlorella vulgaris* found that 25 g/l of copper sulfate did not inhibit respiration if cultures were shaken. If shaking stopped, concentrations greater than 250 mg/l were toxic. Preliminary experiments indicate that lack of air increases toxicity of copper Hale (1937)—according to Jordan, Day, and Hendrixson (1962)—reported that the following concentrations were necessary to control the indicated algae:

0 5 mg/l—*Cladophora*
0 1 mg/l—*Hydrodictyon*
0 12 mg/l—*Spirogyra*
0 20 mg/l—*Ulothrix*

Calcium and magnesium concentrations are usually not given for algal tests, but it would seem that the concentrations deemed safe for fish would also be acceptable for plankton.

Recommendation: The maximum copper (expressed as Cu) concentration (not including copper attached to silt particles or in stable organic combination) at any time or place should not be greater than 1/10 the 96-hour TL_m value, nor should any 24-hour average concentration exceed 1/30 of the 96-hour TL_m value

Cadmium: Few studies have been made of the toxicity of cadmium in the aquatic environment. Mammalian studies have shown it to have substantial cumulative effects. Permissible levels in drinking water are 0 01 mg/l (USDHEW, 1962b), and concentrations of a few μg/g in food (McKee and Wolf, 1963) have caused sickness in human beings. Mount (1967) found accumulations in living bluegills as high as 100 μg/g (dry weight) and in the gills of dead catfish up to 1000 μg/g. Little accumulation was found in the muscle. Consideration should be given to acceptable residue levels in fish when establishing cadmium criteria.

Daphnia appears to be very sensitive to cadmium (Anderson, 1950). Bringmann and Kuhn (1959a) indicate *Scenedesmus*, and *Escherichia coli* are about equally sensitive. Data as yet unpublished (Pickering, in press) reveal that following prolonged exposure there is a large accumulation of cadmium in fish. Even though very little data are available yet, the evidence warrants a more restrictive requirement for cadmium than specified under the general bioassay section.

Recommendation: The concentration of cadmium must not exceed 1/30 of the 96-hour TL_m concentration at any time or place and the maximum 24-hour average concentration should not exceed 1/100 of the 96-hour TL_m concentration.

Hexavalent Chromium: The chronic toxicity of hexavalent chromium to fish has been studied by Olson (1958) and Olson and Foster (1956, 1957). Their data demonstrate a pronounced cumulative toxicity of chromium to trout and salmon Mr. P. A. Olson (personal communication) of Battelle Memorial Institute advises that some recent comparisons of 48 and 96-hour TL_m concentrations with concentrations not adversely affecting the same species indicate that the application factor for hexavalent chromium is 15/100 000 for salmon and 6/100,000 for rainbow trout. He also feels however, that such factors are not valid for carp. Doudoroff and Katz (1953) found that bluegills tolerated a 45 mg/l level for 20 days in hard water. Cairns (1956), using chromic oxide (CrO_3), found that a concentration of 104 mg/l was toxic in 6 to 84 hours *Daphnia* and *Microregma* exhibit threshold effects at hexavalent chromium levels of 0 016 to 0 7 mg/l.

Some data are available concerning the toxicity of chromium to algae. The concentrations of chromium that inhibit growth (Hervey, 1949) for the test organisms are as follows. Chlorococcales, 3.2 to 6.4 mg/l, Euglenoids, 0 32 to 1 6 mg/l; and diatoms, 0 032 to 0 32, mg/l Chromium at sublethal doses sometimes stimulates algae. Patrick (unpublished data) has studied the effects of temperature on the toxicity of chromium to certain algae. Her findings on the concentrations which reduce population growth by 50 percent are as follows.

Nitzschia linearis—50 percent reduction in growth of population as compared with control (soft water 44 mg/l Ca-Mg as $CaCO_3$)

22 C—0 208 mg/l Cr
28 C—0.261 mg/l Cr
30 C—0.272 mg/l Cr

Navicula seminulum var. hustedtii (hard water 170 mg/l Ca-Mg as $CaCO_3$)

22 C—0.254 mg/l Cr
28 C—0 343 mg/l Cr
30 C—0.433 mg/l Cr

Recommendation: Data are too incomplete to do more than urge caution in the discharge of chromium Concentrations of 0.02 mg/l in soft water have been found safe for salmonid fishes.

TABLE III–5A. Pesticides *

INSECTICIDES

[48-hour TL_m values from static bioassay, in micrograms per liter. Exceptions are noted]

Pesticide	Stream Invertebrate[1] Species	TL_m	Cladocerans[2] Species	TL_m	Fish[3] Species	TL_m	Gammarus lacustris,[4] TL_m
Abate	Pteronarcys californica.	100			Brook trout	1,500	640
Aldrin[5]	P. californica	8	Daphnia pulex	28	Rainbow trout.	3	12,000
Allethrin	P. californica	28	D. pulex	21	do	19	20
Azodrin					do	7,000	
Aramite			D magna	345	Bluegill	35	100
Baygon[5]	P. californica	110			Fathead	25	50
Baytex[5]	P. californica	130	Simocephalus serrulatus.	3.1	Brown t	80	70
Benzene hexachloride (lindane).	P. californica	8	D. pulex	460	Rainbow t	18	88
Bidrin	P. californica	1,900	D. pulex	600	do	8,000	790
Carbaryl (sevin)	P. californica	1 3	D pulex	6 4	Brown t	1,500	22
Carbophenothion (trithion).			D. magna	0 009	Bluegill	225	28
Chlordane[5]	P. californica	55	S serrulatus	20	Rainbow t	10	80
Chlorobenzilate			S. serrulatus	550	do	710	
Chlorthion			D magna	4 5			
Coumaphos			D magna	1			0.14
Cryolite			D pulex	5,000	Rainbow t.	47,000	
Cyclethrin			D. magna	55			
DDD (TDE)[5]	P. californica	1,100	D pulex	3 2	Rainbow t	9	1 8
DDT[5]	P californica	19	D pulex	0 36	Bass	2 1	2 1
Delnav (dioxathion)					Bluegill	14	690
Delmeton (systex)				14	do	81	
Diazinon[5]	P. californica	60	D pulex	0 9	do	30	500
Dibrom (naled)	P californica	16	D. pulex	3 5	Brook t.	78	160
Dieldrin[5]	P californica	1.3	D pulex	240	Bluegill	3 4	1,000
Dilan			D magna	21	do	16	600
Dimethoate (cygon).	P californica	140	D magna	2,500	do	9,600	400
Dimethrin					Rainbow t.	700	
Dichlorvos[5] (DDVP)	P. californica	10	D pulex	0 07	Bluegill	700	1
Disulfoten (di-syston)	P. californica	18			do	40	70
Dursban	Peteronareella badia	1 8			Rainbow t	20	0.4
Endosulfan (thiodan)	P californica	5 6	D magna	240	do	1 2	64
Endrin[5]	P californica	0 8	D pulex	20	Bluegill	0 2	4 7
EPH			D. magna	0 1	do	17	36
Ethion	P californica	14	D magna	0 01	do	230	3 2
Ethyl guthion[5]			D pulex		Rainbow t		
Fenthion	P californica	39	D pulex	4			
Guthion[5]	P californica	8	D magna	0 2	Rainbow t	10	0.3
Heptachlor[5]	P. badia	4	D. pulex	42	do	9	100
Kelthane (dicofel)	P. californica	3,000	D. magna	390	do	100	
Kepone					do	37.5	
Malathion[5]	P badia	6	D pulex	1.8	Brook t.	19.5	1.8
Methoxychlor[5]	P. californica	8	D pulex	0 8	Rainbow t	7 2	1 3
Methyl parathion[5]			D magna	4 8	Bluegill	8,000	
Morestan	P californica	40			do	96	
Ovex	P californica	1,500			do	700	
Paradichlorobenzene					Rainbow t.	880	
Parathion[5]	P californica	11	D pulex	0 4	Bluegill	47	6
Perthane			D magna	9 4	Rainbow t	7	
Phosdrin[5]	P. californica	9	D pulex	0 16	do	17	310
Phosphamidon	P californica	460	D. magna	4	do	8,000	3 8
Pyrethrins	P californica	64	D pulex	25	do	54	18
Rotenone	P californica	900	D. pulex	10	Bluegill	22	350
Strobane[5]	P. californica	7			Rainbow t	2 5	
Tetradifon (tedion)					Bluegill	1,100	140
TEPP[5]					Fathead	390	52
Thanite			D magna	450			
Thimet					Bluegill	5 5	70
Toxaphene[5]	P. californica	7	D pulex	15	Rainbow t	2 8	70
Trichlorofon (dipterex).[5]	P. badia	22	D magna	8 1	do	160	60
Zectran	P californica	16	D pulex	10	do	8,000	76

* See notes following Table III-5B

Pesticides

A general description of the use and the effects of pesticides on aquatic life is given in the marine section. Basically, their effects are similar in both the marine and fresh water environments

The addition of any persistent chlorinated hydrocarbon pesticides is likely to result in damage to aquatic life Therefore, as concentrations of these chemicals increase in the aquatic environment, progressive damage will result The acute effects usually will be recognized, but the chronic consequences may not be observed for some time.

The use of other kinds of chemical pesticides in or around fresh waters may produce a variety of acute and chronic effects on fish and the other components of the biota. Because these other chemicals are usually not as persistent as the chlorinated hydrocarbons, the Subcommittee feels some of them can be used around water, but only in amounts below those that produce chronic damage to desirable species.

Recommendation: (1) *Chlorinated hydrocarbons*—Since any addition of persistent chlorinated hydrocarbon insecticides in likely to result in permanent damage to aquatic populations, their use should be avoided

(2) *Other chemical pesticides*—Addition of other kinds of chemicals used as insecticides, herbicides, fungicides, defoliants, acaracides, algicides, etc, can result in damage to some organisms Table III–5 lists the 48-hour TL_m values for a number of pesticides for various types of fresh water organisms To provide reasonably safe concentrations of these materials in receiving waters, application factors ranging from $\frac{1}{10}$ to $\frac{1}{100}$ should be used with these values depending on the characteristic of the pesticide in question and used as specified earlier in the section on application factors Concentrations thus derived tentatively may be considered safe under the environmental conditions recommended

Other Toxic Substances

Detergents and Surfactants: The toxicity of ABS has been reported by many workers A wide range of endpoints have been used as criteria and while comparison is difficult, a reasonable conclusion is possible There is no agreement on the effect of calcium and magnesium concentration. Recommendations are based on the data from table III–6.

Recommendation: With continuous exposure, the concentration of ABS should not exceed $\frac{1}{7}$ of the 48-hour TL_m concentration Concentrations as high as 1 mg/l may be tolerated infrequently for periods not exceeding 24 hours. ABS may increase the toxicity of other materials

Much less work has been done on LAS, a newer, degradable detergent, than on ABS. Bardach, Fujiya, and Holl (1965) report that 10 mg/l is lethal to bullheads and 0.5 mg/l will erode 50 percent of the taste buds within 24 days. For fathead minnow fry, Pickering (1966) reports a 9-day TL_m of 2 3 mg/l. Thatcher and Santner (1967) report 96-hour TL_m values from 3.3 to 6.4 mg/l for five fish species. Swisher, O'Rourke, and Tomlinson (1954), testing bluegills, found TL_m values of 3 mg/l for LAS and 12 carbon homologs and 0.6 mg/l for 14 other carbon homologs An intermediate degradation product had a TL_m of 75 mg/l. Dugan (1967) found that sensitivity to chlorinated pesticides possibly increased after exposure to detergent Other studies as yet unpublished indicate a surprising increase in toxicity at low dissolved oxygen concentrations.

Pickering and Thatcher (in press), in the only reported study on reproduction, found that 0 6 mg/l had no measurable effect on reproduction or growth but 1 1 mg/l had an effect. In tests with five species of fishes Thatcher and Santner (1967) found two species which were more sensitive to LAS than fathead minnows

With both ABS and LAS detergents, the more readily degradable components are the more toxic. As a result, the components remaining will be less toxic than the original product.

Recommendation: The concentration of LAS should not exceed 0 2 mg/l of $\frac{1}{7}$ of the 48-hour TL_m concentration, whichever is the lower

Cyanide: Although it has been studied extensively, cyanide is not well understood as a hazard to aquatic life Certain unique and peculiar characteristics necessitate special treatment of this chemical even though acceptable concentrations cannot be given

Recent work on fish by Doudoroff et al. (1966), has demonstrated that HCN rather than CN is the toxic component. Except for certain extremely toxic heavy metals (silver, for example) the toxicity of metallo-cyanide complexes can also be attributed to the HCN. This then makes the effect of pH on cyanide toxicity of great importance. Doudoroff (1956) demonstrated a thousandfold increase in the toxicity of a nickelo-cyanide complex associated with a drop in pH from 8 0 to 6 5. A change in pH from 7 8 to 7.5 increases the toxicity ten times The data reported by Cairns and Scheier (1963b) indicate that the calcium-magnesium concentrations (hardness) do not materially affect cyanide toxicity It should be noted that in their test solutions while the calcium-magnesium concentration of their soft and hard

TABLE III-5B. Pesticides, cont.

HERBICIDES, FUNGICIDES, DEFOLIANTS, ALGICIDES

Pesticide	Stream invertebrate [1] Species	TLm	Cladocerans [2] Species	TLm	Fish [3] Species	TLm	Gammarus lacustris,[4] TLm
Ametryne	------	------	------	------	Rainbow t	3,400	------
Aminotriazole	------	------	------	------	------	------	------
Aquathol	------	------	------	------	Bluegill	257	------
Atrazine	------	------	Daphnia magna	3,600	Rainbow t	12,600	------
Azide, potassium	------	------	------	------	Bluegill	1,400	10,000
Azide, sodium	------	------	------	------	do	980	9,000
Copper chloride	------	------	------	------	do	1,100	------
Copper sulfate	------	------	------	------	do	150	------
Dichlobenil	Pteronarcys californica.	44,000	Daphnia pulex	3,700	do	20,000	1,500
2,4-D, PGBEE	------	------	D pulex	3,200	Rainbow t.	960	1,800
2,4-D, BEE	P californica	1,800	------	------	Bluegill	2,100	760
2,4 D, isopropyl	------	------	------	------	do	800	------
2,4-D, butyl ester	------	------	------	------	do	1,300	------
2,4-D, butyl + isopropyl ester	------	------	------	------	do	1,500	------
2,4,5-T isooctyl ester	------	------	------	------	do	16,700	------
2,4,5-T isopropyl ester	------	------	------	------	do	1,700	------
2,4,5-T PGBE	------	------	------	------	do	560	------
2(2,4-DP) BEE	------	------	------	------	do	1,100	------
Dalapon	P californica Very low toxicity	------	D magna	6,000	Very Low toxicity		
Dead X	P californica	5,000	D pulex	3,700	Rainbow t	9,400	5,600
DEF	P californica	2,300	------	------	Bluegill	36	230
Dexon	P californica	42,000	------	------	Bluegill	23,000	6,000
Dicamba	------	------	------	------	non tox	------	5,800
Dichlone	------	------	D magna	26	Rainbow t.	48	11,500
Difolitan	P californica	150	------	------	Channel Cat.	31	6,500
Dinitrocresol	P californica	560	------	------	Rainbow t	210	------
Diquat	------	------	------	------	do	12,300	------
Diuron	P californica	2,800	D pulex	1,400	do	4,300	380
Du-ter	------	------	------	------	Bluegill	33	------
Dyrene	------	------	D magna	490	------	15	------
Endothal, copper	------	------	------	------	Rainbow t	290	------
Endothal, dimethylamine	------	------	------	------	do	1,150	------
Fenac, acid	P californica	70,000	------	------	do	16,500	------
Fenac, sodium	P californica	80,000	D pulex	4,500	do	7,500	18,000
Hydram (molinate)	P californica	3,500	------	------	do	290	------
Hydrothol 191	------	------	------	------	do	690	1,000
Lanstan (korax)	------	------	------	------	do	100	5,500
LFN	------	------	------	------	do	79	------
Paraquat	P californica Very low toxicity	------	D pulex	3,700	Very low toxicity	------	18,000
Propazine	------	------	------	------	Rainbow t	7,800	------
Silvex, PGBEE	------	------	D. pulex	2,000	do	650	------
Silvex, isoctyl	------	------	------	------	Bluegill	1,400	------
Silvex, BEE	------	------	------	------	do	1,200	------
Simazine	P californica	50,000	------	------	Rainbow t	5,000	21,000
Sodium arsenite	P californica Very low toxicity	------	Simocephalus serrulatus	1,400	do	36,500	------
Tordon (picloram)	------	------	------	------	do	2,500	48,000
Trifuralin	P californica	4,200	D pulex	240	do	11	5,600
Vernam [5] (vernolate)	------	------	------	------	do	5,900	25,000

[1] Stonefly bioassay was done at Denver, Colo, and at Salt Lake City Utah Denver tests were in soft water (35 mg/l TDS), non-aerated, 60 F Salt Lake City tests were in hard water (150 mg/l TDS), aerated, 48–50 F Response was death

[2] Daphnia pulex and Simocephalus serrulatus bioassay was done at Denver, Colo, in soft water (35 mg/l TDS), non aerated, 60 F Daphnia magna bioassay was done at Pennsylvania State University in hard water (146 mg/l TDS), non-aerated, 68 F Response was immobilization

[3] Fish bioassay was done at Denver, Colo, and at Rome, N Y Denver tests were with 2 inch fish in soft water (35 mg/l TDS), non aerated, trout at 55 F, other species at 65 F Rome tests were with 2–2½ inch fish in soft water (6 mg/l TA pH 5 85–6 4), 60 F Response was death

[4] Gammarus bioassay was done at Denver, Colo, in soft water (35 mg/l TDS), non-aerated, 60 F Response was death

[5] Becomes bound to soil when used according to directions, but highly toxic (reflected in numbers) when added directly to water

water was greatly different, the pH and toxicity were similar.

Burdick and Lipschuetz (1950) show that some metallo-cyanide complexes decompose in sunlight and become highly toxic due to release of cyanide from the complex. Cairns and Scheier (1963b) found some increase in toxicity at reduced oxygen concentrations and Henderson, et al. (1961) demonstrated marked cumulative toxicity of an organic cyanide in 30-day tests.

The toxicity of cyanide to diatoms varied little with change of temperature and was a little more

TABLE III–6. Effect of Alkyl-Aryl Sulfonate, Including ABS, on Aquatic Organisms

Organisms	Concentration (mg/l)	Time	Effect	References
Trout	5.0	26 to 30 hours	Death	Wurtz-Arlet, 1960.
	3.7	24 hours	TL_m	
	5.0		Gill pathology	Schmid and Mann, 1961
Bluegills	4.2	24 hours	TL_m	Turnbull, et al, 1954.
	3.7	48 hours	TL_m	
	0.86		Safe	
	16.0	30 days	TL_m	Lemke and Mount, 1963.
	5.6	90 days	Gill damage	Cairns and Scheier, 1963
	17.0	96 hours	TL_m	
Fathead minnows	2.3		Reduced spawning	Pickering, 1966
	13.0	96 hours	TL_m	Henderson, et al, 1959.
	11.3	96 hours	TL_m	Thatcher, 1966
Fathead minnow fry	3.1	7 days	TL_m	Pickering, 1966.
Pumpkinseed sunfish	9.8	3 months	Gill damage	Cairns and Scheier, 1964.
Salmon	5.6	3 days	Mortality	Holland, et al., 1960.
Yellow bullheads	1.0	10 days	Histopathology	Bardach, et al, 1965.
Emerald shiner	7.4	96 hours	TL_m	Thatcher, 1966.
Bluntnose minnow	7.7	96 hours	TL_m	Thatcher, 1966.
Stoneroller	8.9	96 hours	TL_m	Thatcher, 1966
Silver jaw	9.2	96 hours	TL_m	Thatcher, 1966.
Rosefin	9.5	96 hours	TL_m	Thatcher, 1966.
Common shiner	17.0	96 hours	TL_m	Thatcher, 1966.
Carp	18.0	96 hours	TL_m	Thatcher, 1966.
Black bullhead	22.0	96 hours	TL_m	Thatcher, 1966
"Fish"	6.5		Min lethality	Leclerc and Devlaminck, 1952
Trout sperm	10.0		Damage	Mann and Schmid, 1961.
Daphnia	5.0	96 hours	TL_m	Sierp and Thiele, 1954.
	20.0	24 hours	TL_m	Godzch, 1961.
	7.5	96 hours	TL_m	Godzch, 1961.
Lirceus fontinalis	10.0	14 days	6.7 percent survival (hard water).	Surber and Thatcher, 1963.
Crangonyx setodactylus [1]	10.0	14 days	0 percent survival (hard water).	Surber and Thatcher, 1963.
Stenonema ares	8.0	10 days	20–33 percent survival	Surber and Thatcher, 1963.
	16.0	10 days	0 percent survival	Surber and Thatcher, 1963.
Stenonema heterotarsale	8.0	10 days	40 percent survival	Surber and Thatcher, 1963.
	16.0	10 days	0 percent survival	Surber and Thatcher, 1963.
Isonychia bicolor	8.0	9 days	0 percent survival	Surber and Thatcher, 1963.
Hydropsychidae (mostly cheumatopsyche)	16.0	12 days	37–43 percent survival	Surber and Thatcher, 1963.
	32.0	12 days	20 percent survival	Surber and Thatcher, 1963.
Orconectes rusticus	16.0	9 days	100 percent survival	Surber and Thatcher, 1963.
	32.0	9 days	0 percent survival	Surber and Thatcher, 1963.
Goniobasis livescens	16.0	12 days	40–80 percent survival	Surber and Thatcher, 1963
	32.0	12 days	0 percent survival	Surber and Thatcher, 1963.
Snail	18.0	96 hours	TL_m	Cairns and Scheier, 1964.
	24.0	96 hours	TL_m	Cairns and Scheier, 1964.
Chlorella	3.6		Slight growth reduction	Maloney, 1966.
Nitzchia linearis	5.8		50 percent reduction in growth in soft water.	Cairns, et al., 1964.
Navicula seminulum	23.0		50 percent reduction in growth in soft water.	Cairns, et al., 1964.

[1] Misidentified originally as Synurella

toxic in soft water than in hard water (Patrick, unpublished data). For *Nitzchia linearis* a 50-percent reduction in growth of the population in soft water (44 mg/l Ca-Mg as $CaCO_3$) occurred as follows: 0 288 mg/l (CN) at 72 F, 0.295 mg/l at 82 F, and 0.277 mg/l at 86 F For *Navicula seminulum* var. hustedtii, the concentrations that reduced growth of the population 50 percent in hard water (170 mg/l Ca-Mg as $CaCO_3$) were as follows 0 356 mg/l at 72 F, 0 491 mg/l at 82 F, and 0 424 mg/l at 86 F.

Recommendation: Permissible concentrations of cyanide should be determined by the flow-through bioassay method described in the bioassay section These tests should be conducted with DO, temperature, and pH at recommended levels for the factors under which the cyanide (HCN) is most toxic or under local water conditions at which it is the most toxic.

Ammonia: The toxicity of ammonia has been studied by several investigators but because of inadequate reporting and unsatisfactory experimental control, much of the work is not usable Doudoroff and Katz (1950), Wuhrmann, et al. (1947), and Wuhrmann and Woker (1948) give a complete account of the pH effect on ammonia toxicity and demonstrate that toxicity is dependent primarily on undissociated NH_4OH and nonionic ammonia. They found no obvious relationship between time until loss of equilibrium and total ammonium content. They also demonstrated a striking synergy between ammonia and cyanide. McKee and Wolf (1963) state that toxicity is increased markedly by reduced dissolved oxygen. Field studies by Ellis (1940) and other observations lead to the conclusion that at pH levels of 8 0 and above total ammonia expressed as N should not exceed 1 5 mg/l It has been found that 2.5 mg/l total ammonia expressed as N is acutely toxic.

Recommendation: Permissible concentrations of ammonia should be determined by the flow-through bioassay with the pH of the test solution maintained at 8 5, DO concentrations between 4 and 5 mg/l, and temperatures near the upper allowable levels

Others: Especially significant sources of wastes that must be considered individually are derived from tar, gas, and coke-producing plants, pulp and paper mills, petroleum refining and petrochemical plants, waterfront boating activities, and special-purpose laboratories. These problems are discussed in the toxicity portion of the section on water quality requirements for marine organisms.

marine and estuarine organisms

ESTUARIES are recognized as being of critical importance in man's harvest of economically useful living marine resources It is in these areas that the maximum conversion of solar energy into aquatic plant life takes place and they are justly identified as "nurseries" since so many animals utilize them for feeding their early life stages. Some species, such as the oyster, spend their entire life span in the estuary, while the shrimp and menhaden reside there only as juveniles. The salmon and a few others use the estuary primarily as a pathway In sum, however, more than half of the over 4 5 billion pounds of fishery products harvested by U.S. fisherman annually is derived from animals dependent for their existence on clean estuarine waters during some part or all of their life cycle.

Pollution of estuarine and coastal waters is difficult to assess because of the special qualities of this environment Technically, any foreign substance or environmental condition that interferes with a desired use may be considered a pollutant, but we are concerned with those substances present at high enough concentrations or environmental changes great enough to cause deleterious effect Many naturally occurring substances in salt water become toxic when their concentrations are increased artificially or by natural processes

The problem in establishing criteria in estuaries arises from the fluctuating nature of the water quality, both daily and seasonally, and geographically within the estuary Changes in salinity, pH, turbidity, and temperature may alter greatly the critical toxic concentration of a pollutant Most chlorinated hydrocarbon pesticides, for example, are significantly more acutely toxic at summer rather than winter water temperatures and at least one of the common detergents becomes decidedly more toxic to fish as salinity levels increase

The most obvious effect of tidal action in the estuary is to change water depth This indirectly changes current patterns, water temperature, and the density of motile animal populations Depending on the geography of the estuary and the amount of fresh water drainage into it, salinity patterns may vary from relatively uniform conditions throughout a tidal cycle to situations in which the water is clearly stratified with a layer of relatively fresh water overlying the bottom salt water, or to situations in which the major portion of the water mass changes from fresh to salt and back to fresh again

In shallow, broad estuaries, wind may be the dominant factor in causing water movements which change salinity and temperature patterns. The volume of fresh water discharged into an estuary may be a major factor in establishing coastal currents that transport pollution loads from one region to another.

We are dealing, then, with an environment in which the characteristics of the receiving water are usually fluctuating, frequently unexpectedly. As a result, its ability to dilute and disperse a burden of toxicants is unpredictable without detailed local investigations

Pollution in the estuary may be derived from contamination hundreds of miles upstream in the river basin or it may be of purely local origin Silt plays a major role in the transport of toxicants, especially pesticides, down to the estuary. Agricultural chemicals are adsorbed on silt particles Under poor farming practices, as much as 11 tons of silt per acre per year may be washed by surface water into a drainage basin. Surface mining and deforestation further accelerate the process of erosion and permit the transport of terrestrial chemical deposits to the marine environment

Atmospheric drift is also an important factor in the transport of pollutants to the aquatic environment (Cohen and Pinkerton, 1966) Much of the tonnage of aerially applied pesticides fails to reach the designated spray areas and the presence of 5 $\mu g/l$ of DDT in presumably untreated Alaskan rivers indicates the magnitude of this facet of the pollution problem The continuous presence of 5 $\mu g/l$ of DDT in the marine environment would decrease the growth of oyster populations by nearly 50 percent

Toxic pollutants may be passed directly into the marine environment as contaminants of industrial and domestic waste effluents or they may be intentionally placed there as in the control of various noxious insects by spraying marsh and littoral habitats with synthetic pesticides. Experimentally, some synthetic insecticides have been applied directly to estuarine bottoms in efforts to control oyster pests.

Finally, there are naturally occurring substances such as lignins and phosphate compounds which in times of flood may be carried to the estuary in sufficient quantity to constitute a pollution hazard.

Salinity

The spatial and temporal distribution of salinity profoundly affects the activities of many estuarine species in tidal tributaries (Andrews, 1964; Emery and Stevenson, 1957, Hargis, 1965 and 1966; Pearse and Gunter, 1957, Pritchard, 1953). Some bottom organisms, e g , *Crassostrea virginica,* are able to survive lower salinities than can their predators and disease-causing organisms. Hence, in some tidal tributaries, oysters thrive in regions where they are sheltered from these pests by low salinity. Natural alterations in salinity distribution have been reportedly followed by increased mortality of oysters. It is clear that care must be exercised in the approval of engineering projects or industrial processes that will alter salinity regimes in tidal tributaries and lagoons and in their associated wetlands

Salinity patterns can be caused to vary from "normal" by alterations in character of freshwater inflow and basin geometry. These are the same factors that produce changes in circulation In fact, salinity alterations are precursors to changes in density currents

Recommendation: For the protection of estuarine organisms, no changes in channels, in the basin geometry of the area, or in fresh water inflow should be made that would cause permanent changes in isohaline patterns of more than ±10 percent of the natural variation

Currents

Despite their large volumes, tidal waters, especially those in tributaries of the seas, have special circulatory characteristics that may affect their ability to assimilate wastes For example, tidal action slows the already slowed (due to lowered slope and resulting reduced speed of gravity-induced flow) seaward movement of water in tidal rivers and streams This alternate up and down stream movement of the water in the freshwater portion of the tidal tributary is confounding enough in itself (Ketchum, 1950 and 1951; Stommel, 1953a, b) but in the estuarine reach, the area where sea salts are noticeable, further complexities often occur (Bowden, 1963, Hargis, 1965; Redfield, 1951) In horizontally and vertically stratified mixing estuaries, there are two streams The upper stream, fresher and lighter, has a netflow downstream while the lower stream, saltier and heavier, flows inward or upstream Since these surface currents and bottom counter-currents often extend far to sea off the mouths of large tidal tributary or estuarine systems, as well as far upstream, significant upstream transport of materials in solution or suspension in the countercurrent can occur These circulatory features are important in the life cycles of many estuarine species. For example, oyster and barnacle setting is related to tidal and nontidal currents (Barlow, 1955; Bousfield 1955; Emery and Stevenson, 1957; Hargis, 1966; Ketchum, 1954, Pritchard, 1953). Large disturbances of current patterns can disrupt the life cycles of estuarine organisms. Hence, projects that alter current patterns should be carefully evaluated and controlled.

It is possible to alter circulatory patterns in tidal tributaries by (1) changing the quantity, timing, and location of fresh water inflow, (2) changing the geometry of the basin. The former can be accomplished by construction and operation of reservoirs above or below the fall line (defined as the uppermost limit of ocean's tidal activity). The latter can be accomplished by shoreline or bottom modification; e g, drainage, bulkheading and filling, channel dredging, and subaqueous spoil disposal or mining. Oyster harvesting practices have been known to produce marked changes in bottom geometry (Hargis, 1966)

Recommendation: In view of the requirements of estuarine organisms and the nature of marine waters, no changes in basin geometry or fresh water inflow should be made in tidal tributaries which will alter current patterns in such a way as to cause adverse effects

pH

Despite the great emphasis given to the importance of pH in the literature, little is known of its direct physiological effects on marine organisms. Its indirect effects, however, are extremely significant Even a slight change in pH indicates that the buffering system inherent in sea water has been altered radically and that either a potential or actual carbon dioxide imbalance exists. This imbalance can be deleterious or disastrous to marine life A second indirect effect is that pH can influence the toxicity of other materials Cyanide and ammonia, discussed under "Toxicity," are outstanding examples of this kind of action.

Recommendation: Materials that extend normal ranges of pH at any location by more than ±0 1 pH unit should not be introduced into salt water portions of tidal tributaries or coastal waters At no time should the introduction of foreign materials cause the pH to be less than 6 7 or greater than 8 5

Temperature

Temperature requirements of marine and estuarine organisms in the biota of a given region may vary widely Therefore, if we are to maintain temperature favorable to the biota, all important species, including the most sensitive, must be protected. It has been found that organisms in the intertidal zone vary considerably in their ability to withstand high temperatures Those in the uppermost areas of the tidal zone generally can withstand higher temperatures than those in the lower portions of the tidal zone and these in turn generally can withstand higher temperatures than the same species of animals living in the subtidal zones. In addition, when considering the coastline as a whole, we must recognize that there are various races within a given species which may vary considerably in their environmental requirements, or in their ability to withstand extreme conditions.

In our marine waters, there is a great mixture of species Species typical of higher latitudes are found with species that are more abundant farther south Tropical or subtropical species generally will spawn in the summer months. Species from the higher latitudes require low water temperature for spawning and the development of the young Thus, they usually spawn in the winter months and temperatures at that time are critical Any warming of the water during the cold weather or winter period could be disastrous from the standpoint of the elimination of the more northerly species. In some instances, a rise in winter temperatures of only 2 or 3 F might be sufficient to prevent spawning and thus eliminate these species from the biota

In the northern portions of the country there is generally a great range in natural temperatures In southern areas, as we approach the tropics, we find smaller overall temperature ranges. In the tropics or subtropics, optimum temperatures for many forms are only a few degrees lower than maximum lethal temperatures Great care should be exercised, therefore, to prevent harmful increases in maximum summer temperatures in tropical areas

In general, temperatures in the marine waters do not change as rapidly nor do they have the overall range from extreme to extreme as they do in fresh waters. Marine and estuarine fishes, therefore, are less tolerant of temperature variation. They can accommodate somewhat, but overall temperature range and rate of change are even more important here than they are in fresh waters. It has been observed that when surface water temperatures over the Georges Bank increased from 46 to 68 F, the larval fish died at 65 F. It has been found that species in subtropical and tropical environments are living at temperatures that are only a few degrees less than their lethal temperatures In the most northern forms, extensive variations in seasonal temperatures are a necessity for orderly development and growth. Spawning and development frequently occur at lower temperatures and the sexual products ripen on rising temperatures after a period of low temperatures Temperatures above or below the optimum range may delay or speed up development They may inhibit swimming ability and the effectiveness of food utilization may be decreased with increasing temperatures in the upper viable range. Fishes and other forms are also more susceptible to parasites and diseases at temperatures outside of their optimum range. In regard to rapid changes in temperature, it has been found that a drop in temperature from 58 to 43 F kills sardines. Tolerable temperature minima vary with the population and its past temperature history. Kills have occurred off the Texas coast at 40 F whereas kills of the same species have occurred off Bermuda at a drop to 59 F. Many kills have occurred in nature due to unusually low temperatures. Kills also occur due to natural high temperatures. Yellowtail flounder and whiting larvae died when they drifted from an area of 44 F to one of 64 F It has been reported that 61 F is best for the developing of mackerel, but 70 F is too high These are merely illustrations of what might happen to species occurring in inshore waters

It is apparent from the foregoing that data are very sparse on temperature requirements of marine and estuarine species It is very difficult, therefore, to attempt to suggest temperature requirements for marine and estuarine forms The difficulty is compounded by the great extent of the Nation's shorelines, the differing natural temperature variations from north to south, and the geographic overlapping of species native to different latitudes Consideration must be given to maximum allowable temperatures for both the summer period and the winter period.

In attempting to establish permissible levels of temperature increase in receiving waters due to heated waste discharges, precaution must be taken to prevent—

(a) excessive incremental increases above background values even though such incremental increases lie below maximum limits, and

(b) exceeding maximum natural background limits

Such precautions are necessary to prevent gradual net increases in background temperatures due to the continuously increasing volumes of heated wastes being discharged into receiving waters

The discharge of heated wastes into estuaries and other tidal tributaries must be managed so that no barrier to the movement or migration of fish and other aquatic life is created

Recommendation: In view of the requirements for the well-being and production of marine organisms, it is concluded that the discharge of any heated waste into any coastal or estuarine waters should be closely managed Monthly means of the maximum daily temperatures recorded at the site in question and before the addition of any heat of artificial origin should not be raised by more than 4 F during the fall, winter, and spring (September through May), or by more than 1 5 F during the summer (June through August) North of Long Island and in the waters of the Pacific Northwest (north of California), summer limits apply July through September, and fall, winter, and spring limits apply October through June The rate of temperature change should not exceed 1 F per hour except when due to natural phenomena

Suggested temperatures are to prevail outside of established mixing zones as discussed in the section on zones of passage

Dissolved oxygen

Dissolved oxygen requirements of marine organisms are not as well known as those for freshwater forms Studies have been made indicating that minimum dissolved oxygen concentrations of 0 75 to 2 5 are required for test species to resist death for 24 hours. Most marine species died when the dissolved oxygen dropped below 1.25 mg/l for a few hours. Reduced swimming speed and changes in blood and serum constituents occurred at dissolved oxygen levels of 2.5 to 3 mg/l It was found that DO levels between 5 3 and 8 mg/l were satisfactory for survival and growth Levels above 17 mg/l, however, produced adverse effects Large fluctuations in dissolved oxygen from 3 to 8 mg/l, diurnal or otherwise, produced significantly more physiological stress in fishes than fluctuations from 3 to 6 mg/l In tests made to date, it has been found that 5 to 8 mg/l of DO is apparently sufficient for all species of fish for good growth and general well being It is generally recognized that in deeper waters DO values are often considerably less than 5 0 mg/l In estuaries where there is a reduction in salinity, levels may drop to as low as 4 mg/l at infrequent intervals and for limited periods of time It is probable that many marine animals can live for long periods of time at much lower levels of DO. Experimental studies with freshwater organisms have demonstrated, however, that low concentrations of DO at which adult fishes can live almost indefinitely, can inhibit feeding and growth In determining DO requirements, it is essential to consider growth, reproduction, and other necessary life activities.

Recommendation: For the protection of marine resources, it is essential that oxygen levels shall be sufficient for survival, growth, reproduction, the general well-being, and the production of a suitable crop To attain this objective, it is recommended that dissolved oxygen concentrations in coastal waters, estuaries, and tidal tributaries of the Nation, including Puerto Rico, Alaska and Hawaii, should be as follows.

(1) Surface dissolved oxygen concentrations in coastal waters shall not be less than 5.0 mg/l, except when natural phenomena cause this value to be depressed.

(2) Dissolved oxygen concentrations in estuaries and tidal tributaries shall not be less than 4 0 mg/l at any time or place except in dystrophic waters or where natural conditions cause this value to be depressed

The committee would like to stress the fact that, due to a lack of fundamental information on the DO requirements of marine and estuarine organisms, these requirements are tentative and should be changed when additional data indicate that they are inadequate

Crude oil and petroleum products

The discharge of crude oil and petroleum products into estuarine and coastal waters presents special problems in water pollution abatement. Oils from different sources have highly diverse properties and chemistry Oils are relatively insoluble in sea and brackish waters and surface action spreads the oil in thin surface films of variable thickness, depending on the amount of oil present. Oil, when adsorbed on clay and other particles suspended in the water, forms large, heavy aggregates that sink to the bottom. Additional complications arise from the formation of emulsions in water, leaching of water soluble fractions, and coating and tainting of sedentary animals, rocks, and tidal flats

Principal sources of oil pollution are numerous. Listed in order of their destructiveness to ecosystems, they are.

(1) Sudden and uncontrolled discharge from wells towards the end of drilling operation

(2) Escape from wrecked and submerged oil tankers.

(3) Spillage of oil during loading and unloading operations, leaky barges, and accidents during transport

(4) Discharge of oil-contaminated ballast and bilge water into coastal areas and on the high seas.

(5) Cleaning and flushing of oil tanks at sea. On the average, a ship's content of such wastes is estimated to contain 2 to 3 percent oil in 1,000 to 2,000 tons of waste.

(6) Spillage from various shore installations, refineries, railroads, city dumps, garages, and various industrial plants.

Spillage From Wrecked Oil Tankers

Even though wrecks of oil tankers along the Atlantic coast and subsequent spillage of oil into the sea have been reported several times, no thorough examination has been made of the effect of oil pollution on local marine life, except for frequent references to the destruction of waterfowl One of these disasters attracted the general attention of the public and members of the Audubon Society of New England One night in 1952, two tankers, the *Fort Mercer* and the *Pendleton,* went aground on the shoal of Monomoy Point, Cape Cod. Large amounts of oil spilled from the broken vessels, spread long distances along the shore, and were responsible for high mortality of ducks (scoters and eiders). Many thousands of oil-smeared dying birds were seen along the coast Attempts to save some of the birds by removing the oil with various solvents failed No published records are found on the effect of this massive spillage on aquatic life. According to the records of the Massachusetts Audubon Society, serious oil spreads threatening fish and bird life have occurred at least six times since 1923 along the beaches of Cape Cod The latest occurrence was on Sunday, April 16, 1967. Heavy films of crude oil appeared along the coast from Chatham to Provincetown, Mass. and spread to Cape Cod Bay, Nantucket Island, and Boston. The shores of the National Seashore Park were seriously affected and hundreds of ducks and brant were found dead or dying.

The massive spillage of oil may constitute a disaster of a national and even international magnitude as has been dramatically demonstrated by the wreck in March 1967 of the super tanker *Torrey Canyon* carrying 118,000 tons of crude oil About one-half of the load gradually spilled near Seven Stones Reef, off the southern coast of England, where the tanker was stranded By the middle of April, patches of crude oil began to appear on the French coast in Brittany, threatening the productive oyster farms in the inlets and estuaries It is obvious that a disaster of such magnitude is beyond the scope of an ordinary pollution problem in coastal waters The probability of a recurrence of heavy oil spillage is, however, very real because of the present trend in the methods of transporting oil in very large and apparently vulnerable tankers It has been reported that Japan operates a tanker, *Idemitsu Maru,* of 205,000 tons holding capacity. A super tanker of over 300,000 tons capacity is under consideration and a design of a 500,000-ton tanker appeared in the press

Effect of Oil Spillage on Aquatic Life of a Small Marine Cove

W. J. North, et al (1965) made a valuable study of the effect of massive spillage of crude oil into a small cove in lower California Bay Prior to the spillage, the investigators were engaged in a study of bottom fauna and flora of the cove and were in possession of background information which made it possible for them to record the changes that took place after the water of the cove was contaminated by the 59,000 barrels of oil that escaped from the wreck of the tanker *Tampico* on March 29, 1957. Among the many dead and dying species observed a few weeks after the disaster, the most frequently found were abalones (*Haliotis fulgens, H rufescens,* and especially *H. cracherodii*), lobsters (*Panulirus interruptus*), pismo clams (*Tivela stultorum*), mussels (*Mytilus sp.*), sea urchins (*Strongylocentrotus franciscanus, S purpuratus*), and sea stars (*Pisaster giganteus, P ochraceus*) A slight improvement of the bottom fauna was noticeable a few months after the disaster, but extensive recovery became apparent only 2 years later. Four years after the accident, the populations of abalones and sea urchins still were reduced greatly and seven species of animals previously recorded in the cove had not been found at all.

Combined Effect of Oil and Sewage Pollution

The oil and sewage pollution effects on aquatic organisms of the Novorossiyak Bay (Black Sea, U.S.S.R) was recently studied by Kalugina, et al (1967) For a number of years, this bay has been receiving a mixed daily discharge of 15,000 to 30,000 cubic meters of petroleum refinery wastes and domestic sewage. There is marked decrease of various valuable species of mollusks (*Spisula subtruncata, Tapes rugatus, Pecten ponticus*) and complete destruction of oyster beds (*Ostrea taurica*) due to the combined effect of pollution and depradations by a carnivorous gastropod (*Rapana*) Samples were collected 1 to 25 meters from the outfall for bioassay Copepods (*Acartia clausi*) placed in samples taken 25 meters from the outfall were killed in 24 hours Larvae of decapods and gastropods in samples taken 10 to 25 meters out perished in 3 to 4 days *Calanus* was killed in 5 days in samples taken 1 meter out, but survived the 10-day test in the samples taken 5, 10, and 25 meters from the outfall. There also was a noticeable change in the distribution and species composition of benthic algae.

Color of Oil Film on the Surface of Water

The color of the oil film on the water surface is indicative of the thickness of the slick and may be used as an indicator of the volume of oil spilled.

According to data published by the American Petroleum Institute (1949), the first trace of color that may be observed as a surface film on the sea is formed by 100 gallons of oil spread over 1 square mile. Films of much darker colors may indicate 1,332 gallons of oil per square mile. Experiments conducted by the Committee on the Prevention of Pollution of the Seas (1953) showed that 15 tons of oil covered an area of 8 square miles In 8 days, it had drifted about 20 miles from the point of discharge The same committee (1953) indicated another source of oil pollution that should not be neglected It has been found that unburned fuel oil escaping through the funnels of oil-burning ships may comprise 1 to 2 percent of the total oil consumed and it may be deposited on the sea surface. British investigators attributed the disappearance of eel grass (*Zostera*) to minute quantities of oil. Oil weakens the plant and makes it susceptible to attacks of a parasitic protozoan (*Labyrinthula*). Observations made several years ago at Woods Hole showed that young *Zostera* that began to reappear in local bays after several years of absence were already infected by this microorganism even though they appeared to be healthy

Adsorption of Oil by Sand, Clay, Silt, and Other Suspended Particles

Oil of surface films is easily adsorbed on clay particles and other suspended materials, forming large and relatively heavy aggregates that sink to the bottom The surface of the water may appear free from pollution, until the sediment is stirred by wave action and the released oil floats up again.

During World War II, a product known as "carbonized sand" was manufactured for the U S Navy and used for the primary purpose of rapidly removing oil spilled or leaked from ships Carbonized sand was used principally as a rapid method to prevent and stop fires Sand and oil aggregates, being much heavier than sea water, sank very rapidly and remained on the bottom Experimental work has shown that the toxic effect of oils is not diminished by this method (Chipman and Galtsoff, 1949). Since the end of World War II, a number of preparations to be used as solvents, emulsifiers, and dispersing agents of oil slicks in harbor waters appeared in New Zealand, Western Europe, and the United States These preparations are being offered under various trade names and their chemical composition is not always stated. It is often claimed that such compounds remove oil slicks more efficiently than mopping with straw or coarse canvas fabric (skrim), a method extensively used in Auckland Harbor (Chitty, 1948). It is, however, generally recognized that various detergents and emulsifiers are toxic to aquatic life and therefore compound the danger of oil pollution. Mechanical means such as preventing the spread of a slick by surrounding it with floating barriers (plastic booms), spreading sawdust and removing an oil aggregate by scooping or raking, and erecting grass or straw barriers along the beaches are probably more effective at present than the chemical methods of dispersing or dissolving oil Even anchoring oil by combining it with relatively heavy carbonized sand seems to be preferable to chemical methods.

Toxicity of Crude Oil and Petroleum Products

Oil may injure aquatic life by direct contact with the organism, by poisoning with various water soluble substances that may be leached from oil, or by emulsions of oil which may smear the gills or be swallowed with water and food. A heavy oil film on the water surface may interfere with the exchange of gases and respiration.

A number of observations have been recorded of the concentrations of oil in sea water which are deleterious to various species Experimental data, however, are scarce and consequently the toxicology of oil to marine organisms is not well understood

Nelson (1925) observed marine mollusks (*Mya arenaria*) being destroyed by oil on tidal flats of Staten Island, N.Y. The Pacific coast sea urchin, *Strongylocentrotus purpuratus,* dies in about 1 hour in a 0 1 percent emulsion of diesel oil. After 20 to 40 minutes in this concentration the animals fail to cling to the bottom and may be washed away (North, et al., 1964).

Crude oil absorbed by carbonized sand does not lose its toxicity. This has been shown by laboratory experiments conducted at Woods Hole (Chipman and Galtsoff, 1949). The amount of oil used was limited to the quantity held in the sand, hence no free oil was present in the water. The oil-sand aggregates were placed in containers filled with sea water but never came into contact with the test animals Four species were bioassayed· the very hardy toadfish (*Opsanus tau*) in the yolk sac stage, the moderately tolerant barnacle (*Balanus balanoides*), and oyster (*Crassostrea virginica*), and the extremely sensitive hydrozoan, (*Tubularia crocea*)

The survival of toadfish embryos was indirectly proportional to the concentration of oil in water.

In a concentration of 0 5 percent, the embryos survived for 13 days (end of test); in 1.25 percent, 8½ days; in 2.5 percent, 6 days; and in 5 percent, 4½ days. Barnacles suffered 80 to 90-percent mortality within 70 hours in 2.0-percent mixtures of oil in sea water. They became inactive in 23 hours in concentrations of 2 percent and above. *Tubularia* suffered 90 to 100-percent mortality within 24 hours after being placed in water containing a 1:200 oil-carbonized sand aggregate. Water extracts of crude oil were lethal within 24 hours at concentrations of 500 mg/l and greater.

Experiments with oysters consisted primarily of determining the effect of oil adsorbed on carbonized sand on the number of hours the oysters remain open and feeding and on the rate of water transport, across the gills. A paste-like aggregate of oil in carbonized sand (50 ml crude oil to 127 g sand) was prepared, wiped clean of excess oil, and placed in the mixing chamber. Sea water was delivered through this chamber to the recording apparatus at a rate slightly in excess of the rate of water transport by oyster gills (Galtsoff, 1964; Chipman and Galtsoff, 1949). There was a noticeable decrease in the number of hours the test oysters remained open and in the daily water transport rate through the gills. The time open was reduced from 95 to 100 percent during the first 4 days of testing to only 19 8 percent on the 14th day. The total amount of water transported per day, and presumably used for feeding and respiration, was reduced from 207 to 310 liters during the first 6 days to only 2 9 to 1 liter per day during the period between the eighth and 14th day of continuous testing These tests indicate that oil incorporated into the sediments near oyster beds continues to leach water-soluble substances which depress the normal functions of the mollusk.

Critical observations are lacking on the effect of oil on pelagic larvae of marine invertebrates, but there is good reason to assume that crude oil and petroleum products are highly toxic to free-swimming larvae of oysters Speer (1928) considers that they are killed by contact with surface oil film. Laboratory experience of Galtsoff (unpublished records) shows that oyster larvae from 5 to 6 days old were killed when minor quantities of fuel oil were spilled by ships in the Woods Hole harbor and the contaminated water penetrated into the laboratory sea water supply.

The tests described above leave no doubt that water-soluble substances are leached from oil spilled into water and adversely affect marine life. It is reasonable to assume that the water soluble materials of oil may contain various hydrocarbons, phenols, sulfides, and other substances toxic to aquatic life. The water-soluble fraction leached from crude oil is easily oxidized by aeration and loses its toxicity (Chipman and Galtsoff, 1949).

Carcinogenic Substances From Oil-Polluted Waters

Presence of hydrocarbons similar to benzopyrene in oil-polluted coastal waters and sediments of France in the Mediterranean was reported by Mallet (1965) and Mallet and Sardou (1965). The effluents from the industrial establishments on the shores at Villefranche Bay comprise tar substances, which contain benzopyrenes, benzo-8, 9-fluoranthene, dibenzanthracenes, chrysene, 10-methyl anthracene, and nitrogenous derivatives such as dimethylbenzacridine. These substances are carried out into the bay water and settle on the bottom. The pollution is augmented by incompletely burned oils discharged by turbine ships. The content of benzopyrene in bottom sediments ranges from 500 micrograms in 100 g sample collected at the depth of 8 to 13 cm to 1 6 micrograms at 200 cm. Similar contamination is of importance in the Gulf of Fos, Etang de Berne, and in the delta of the Rhone River.

Carcinogenic hydrocarbons were found to be stored in plankton of the bay of Villefranche, in concentrations varying from 2 5 to 40 micrograms per 100 g. Fixation of benzopyrenes was found also in the bodies of holothurians (Lalou, 1965) in a bay near Antibes. The reported concentration in the visceral mass of holothurian was slightly higher than that in the bottom sediment.

Observations on storage of carcinogenic compounds found in oil-polluted water are biologically significant. The important question of biological magnification as these compounds are ingested by plankton feeders remains unanswered and needs to be investigated.

Sampling of Oil-Polluted Sea Water

The question of the minimal concentration of oil and petroleum products consistent with uninhibited growth and reproduction of aquatic species is more difficult to answer than it is in the case of other contaminants. As has been shown above, oil is found in water in four distinct phases: (1) surface oil film, (2) emulsion in sea water, (3) extract of water soluble substances, and (4) semisolid aggregate of oil and sediment covering the bottom. Obviously, no single sample could include all four phases and the method of sampling should vary accordingly. Collection of samples of a heavy oil slick near the origin of spillage presents no particular difficulty because an adequate quantity may be scooped easily and placed in a proper container. Serious difficulty arises, however, in case of an irridescent film of oil approaching the thickness of a monomolecular layer Garrett (1964), made a special study of slick-forming materials naturally occurring on sea surfaces and demonstrated their highly complex composition. The collection of very thin layers of surface water was made by means of a specially constructed plastic screen The entrapped compounds were washed off into a large container (Garrett, 1962) He found surface-acting substances in all areas where the sea surface was altered by monomolecular films and concluded that "a chemical potential exists whereby such surface alterations can occur when conditions are suitable for the adsorption and compression of the surface-active molecules at the air/water boundary." The oil film at the air/water boundary may be composed of several interacting organic compounds. This complexity must be kept in mind in studies of oil pollution in sea water

If a relatively thick layer of contaminated water is needed, the sample may be scooped or sucked from an area of sea surface enclosed by a floating frame Interference due to wave ripples is minimized in this way.

For analysis of an oil emulsion in sea water, a sample of a desired volume may be collected by pump or by any type of self-closing water bottle lowered within the surf area

For obtaining water soluble substances leached from oil sludge, sampling should be made by pumping or by using a water sampler lowered as close as possible to the oil-covered bottom

Samples of oil adsorbed on sediments can be obtained by using bottom samplers designed to take quantitative samples.

Contamination of beaches by floating tar ballast and cleaning water discharged by ships sailing along our coast is of such common occurrence that at present it is almost impossible to find a public beach free from this nuisance. Cakes of solidified oil tar can be picked by hand from the tidal zone of any beach along the Atlantic and Gulf coasts.

Recommendation: Until more information on the chemistry and toxicology of oil in sea water becomes available, the following requirements are recommended for the protection of marine life No oil or petroleum products should be discharged into estuarine or coastal waters in quantities that (1) can be detected as a visible film or sheen, or by odor, (2) cause tainting of fish and/or edible invertebrates, (3) form an oil-sludge deposit on the shores or bottom of the receiving body of water, or (4) become effective toxicants according to the criteria recommended in the "Toxicity" section

Turbidity and color

Turbidity, color, and transparency are closely interrelated phenomena in water They must be observed simultaneously because transparency is a function of turbidity, water color, and spectral quality of transmitted light For practical purposes, however, it is more convenient to discuss them separately.

Turbidity

By observing the turbidity of sea water it is possible to determine the depth of the euphotic zone; i e., the depth in which organic carbon is produced. Various particles suspended in water reduce the intensity of light by absorption and scattering. In the sea, the maximum depth of growth of attached plants varies It is 160 m in the Mediterranean, 30 m in Puget Sound, and 10 m off Cape Cod. In general, benthic plants will not grow at a depth at which the light intensity is less than 0 3 percent of its surface value (Clarke, 1954). In any environment, the rate of photosynthesis decreases with the attenuation of light but the respiration rate remains approximately the same Because the role of phytoplankton in organic production is far more important quantitatively than that of benthic plants, an increase in the turbidity of water diminishes primary productivity of the ocean biomass as indicated by the rate of growth of various planktonic algae

For each species of plant, a level of light intensity may be reached at which the rate of photosynthesis becomes equal to the rate of respiration This level is designated as compensation intensity and the depth at which this value is found is called the compensation depth For marine phytoplankton, it has been determined that compensation intensity is about 100 ft-candles, or 1 percent of the value of full sunlight (Clarke, 1954) In natural waters, the compensation depth varies; e.g , in the Gulf of Maine it was found to be 30 m while at Woods Hole only 7 m

In many coastal waters, the principal cause of turbidity is the discharge of silt carried out by the principal rivers Secchi disc readings show that the transparency of water at the mouths of large rivers during flood stage may be reduced to a few centimeters. At normal river stages, the disc may be visible at several meters below the surface. Observations from an airplane are useful in recording the distribution of brackish, silt-laden waters along the coast. Silting of the estuaries and adjacent coastal water should be considered as a special case of pollution resulting from deforestation, overgrazing and faulty agricultural practices, road construction, and other land management abuses.

Mixed effluents from various industrial plants and domestic sewage increase the turbidity of receiving water. It is difficult to distinguish between the effect of the attenuation of light due to suspended particles and the direct effect of the particles in suspension on the growth and physiology of aquatic organisms. Natural silt taken from the bottom of the sea and kaolin affect the development of eggs and the growth of larvae of oysters and hard shell clams (*Mercenaria mercenaria*) In a suspension of 2 g of dry silt in a liter of sea water, only 39 percent of oyster larvae completed development In 3 g per liter there was no development (Loosanoff, 1962). Growth of *Mercenaria* clams was retarded in the concentration of 1 to 2 g/l, but appeared to be normal at 0.75 g/l. Development was completely suppressed in the concentration of silt from 3 to 4 g/1 (Davis, 1960). Silt concentration of 0 1 g/l caused a 57-percent decrease in the water transport of an adult oyster. In 4 g/l, the depression was 94 percent (Loosanoff, 1962). The turbidity used in these experiments probably is equivalent to 750 to 4,000 mg/l of turbidity standards, although direct comparison of figures cannot be made accurately.

The principal significance of turbidity observations in a study of pollution is the determination of the depth of the euphotic zone as a factor affecting primary productivity of the sea (Ryther, 1963). Determination of the coefficient "k" defined as the natural logarithm of the fraction of incident light penetrating to a given depth is of great importance in studies of organic production. In the temperate and northern parts of the ocean, values of "k" range between 0.10 to 0.20 and correspond to depths of 50 to 25 m In more turbid coastal waters, the coefficient of extinction is as high as 1 0 and a compensation depth of 5 m is commonly encountered. These values may be used as a basis for comparing the characteristics of uncontaminated waters with those of highly turbid and polluted waters of coastal and inshore areas A considerable part of the turbidity of these areas is attributable to nonliving particles

It must remembered, also, that very high turbidity of sea water may be due entirely to blooms such as are known to occur in red tide areas (Galtsoff, 1949) or as a result of unbalanced overfertilization such as is induced by organic wastes from duck farms in Great South Bay, N.Y. Turbidity may be determined practically by use of a Secchi disc Turbidity may be determined more accurately by using the techniques described in Standard Methods for the Examination of Water and Wastewater, 12th edition (1965). Any turbidity of less than 1 m (by Secchi disc) or in corresponding Jackson units should be regarded with suspicion and the nature of suspended material as well as the composition of plankton determined

Color

The color of sea water, expressed as dominant wave length in millimicrons (mμ) covers the range from violet (400 to 465 mμ) to red-purple (530 to 700 mμ) Spectrophotometric methods, as described in Standard Methods for the Examination of Water and Wastewater, 12th edition (1965), should be used if careful study is required, particularly for determining the exact color of water contaminated with industrial wastes.

Monitoring the color changes of sea water yields information on the extent of intrusion of fresh water into the sea, the intensity and extent of silting, the location and extent of plankton blooms, the extent and distribution of pollution from industrial waste effluents, and the presence and probable thickness of oil film.

In brackish waters, the blue hue of the open sea is replaced by a greenish or yellowish color. Silting areas are recognizable by brown or yellowish discoloration Red-brownish color is typical of the red tide caused by *Gymnodinium* and other species of dinoflagellates. Some of these are toxic to fishes and benthic invertebrates (Galtsoff, 1948, 1949). Mass production of forms such as the blue-green alga *Trichodesmium* gives the surface of the sea an appearance of "green meadow" as described for the Azov Sea by Knipowich (Galtsoff, 1949). Swarming of *Phaeocystis pouchetii, P. globosa,* and *Rhizosolenia* have been reported to extend over hundreds of square miles of the open sea causing a distinct brownish discoloration.

Systematic studies have not been made yet to determine the optical characteristics of discolored sea water. It is reasonable to expect that such an investigation would be valuable in explaining the cause of discoloration and, in certain instances, may indicate the presence and nature of pollution. Light components specific for the contaminant entering sea water may be detected by the use of a spectrophotometer or with the recording SPOT spectroradiometer recently developed by Alfred C. Konrad of the Massachusetts Institute of Technology. This type of instrument is being used at present at the Woods Hole Oceanographic Institution and is proving very useful. Spectroradiometer observations can be made either from an airplane or from shipboard.

Recommendation: No effluent which may cause changes in turbidity or color should be added to, or discharged into, inshore or coastal waters unless it has been shown that it will not be deleterious to aquatic biota.

Settleable and floating substances

Settleable solids entering coastal waters include various products of forest industries such as sawdust, bark chips, wood fibers, sewage solids, and many industrial wastes. The old practice of dumping sawdust into tidal rivers was discontinued long ago, but its effect is still visible in the rivers of Maine For instance, an area of the bottom of the Damariscotta River was still covered with a loose layer of sawdust about 2 to 3 feet deep in 1940, although operation of the lumber mills responsible for this deposition had ceased more than 50 years previous The Damariscotta kitchen-midden on the banks of the river contains a huge accumulation of river oyster shells and some artifacts left here by the Indians who lived there for several centuries of pre-Colonial times The habitat was so completely changed by pollution that at present there is hardly any benthic organism found on this formerly productive bottom (Galtsoff and Chipman, unpublished report).

Decay-resisting organic matter from wood fibers and waterlogged bark and chips constitutes, in places, a serious handicap to aquatic life. Settleable materials from mining operations and gravel and sand washing make the bottom unsuitable for aquatic life in the affected areas of the receiving bodies of water. Silting may be so heavy that the sediment brought in may completely fill the bay One can see this in the eastern branch of Matagorda Bay, Tex., an area that has been completely obliterated within the last 25 years by the Colorado River.

Dredging of bays and tidal rivers for improvement of navigation occasionally presents serious problems Benthic communities in the area near dredging operations may be destroyed or damaged by spoil deposition, increase in water turbidity, release of toxic substances accumulated in the mud of the polluted areas, and by changing the pattern of currents in the dredged area.

Careful studies of the effects of dredging on oyster-producing bottoms of the Santee River, S C, were made in 1936 by G. Robert Lunz, Jr (unpublished report), for the U.S Corps of Engineers No deleterious effect on oyster-producing bottoms was found. An examination made by the Bureau of Fisheries Laboratory at Woods Hole of dredging operations to deepen and enlarge the Cape Cod Canal disclosed that several productive oyster beds near the site of dredging were covered by 2 to 3 feet of sand and silt. The oysters were destroyed, but the grounds soon were re-populated by hard-shell clams and the productivity of the area restored.

Disposal of the huge quantities of garbage accumulated by large cities presents a special and difficult problem. The old practice of barging this waste out to sea and dumping it is highly objectionable Incineration seems to be the answer. This creates, however, the problem of proper incineration of large quantities of materials without increasing air pollution over the metropolis The city of Boston disposes of large amounts of accumulated garbage and trash by incineration and by dumping the ashes into the sea at a distance from shore State and Federal authorities are engaged presently in a study of the chemical composition of ash and its possible effect on aquatic life in the sea Preliminary analysis of an incinerated sample made by Ronald Eisler (personal communication) of the National Marine Quality Laboratory of the Federal Water Pollution Control Administration shows that aluminum, iron, and calcium were most abundant, followed by zinc, sodium, potassium, and lead. Other metals comprising more than 1 percent of the fraction soluble in 6NHCl include barium, chromium, and magnesium It is evident that ash from this waste contains a fairly large percentage of heavy metals which may be accumulating in the bodies of fish and shellfish The effect of ash on the behavior of fish is now being studied, but the results are not yet available.

Examples of industrial effluents containing materials that precipitate in sea water are the waste from titanium paint plants or the soap portion of the effluents from Kraft pulp mills. This fraction of the black liquor is precipitated from solution by salt, carried by the current of the receiving river, and eventually deposited on the bottom (Galtsoff, et al, 1947). Waste from several plants extracting titanium dioxide from ilemenite (ferrous titanate) produces serious pollution in the lower Patapsco River area near Baltimore. Because of the restricted circulation of water in the upper Chesapeake Bay, the effect is quite pronounced. Ferric hydroxide flocculation in the Patapsco River has been found detrimental to plankton. Diatoms were destroyed by flocculation and removed from plankton by settling with the iron particles. Considerable amounts of iron accumulated on the bottom and iron precipitate was found coating the gills of minnows, silverside, and white perch (Olson, et al., 1941).

Recommendation: Water quality requirements for specifying the permissible limits of settleable solids and floating materials cannot be expressed quantitatively at present Since it is known that even minor deposits may reduce productivity and alter the benthic environment, it is recommended that no materials containing settleable solids or substances that may precipitate out in quantities that adversely affect the biota should be introduced into estuarine or coastal waters It is especially urgent that areas serving as habitat or nursery grounds for commercially important species (scallops, lobsters, oysters, clams, crabs, shrimp, halibut, flounders, demersal fish eggs and larvae, and other bottom forms) be protected from any infringement on natural conditions

Tainting Substances

Substances found in industrial wastes are frequently responsible for objectionable or offensive tastes, odors, and colors of fish and shellfish Even slight amounts of oil or petroleum products in bays and estuaries will impart an oil or kerosene flavor to mullet, mackerel, and other fishes and also to oysters, clams, and mussels making them unmarketable Oysters collected in Louisiana waters polluted by crude oil retained a distinct flavor and odor associated with this type of pollution for several weeks after the escape of crude oil from wells and leaky barges had been stopped (Galtsoff, et al, 1935).

Anaerobic conditions associated with the deposit of sewage sludge on the bottom are accompanied by the production of hydrogen sulfide, a substance that causes black discoloration of bivalve shells and imparts an offensive flavor and odor to their flesh In the waters receiving black liquor from Kraft pulp mills in the York River, Va., the gills and mantles of oysters developed a gray color. This condition also is found in oysters grown in waters receiving domestic sewage (Galtsoff, et al, 1957).

Contamination of water with copper results in the accumulation and storage of this metal far above its normal content in the tissues. The copper content of oyster flesh from uncontaminated waters off Cape Cod varied from 0 170 to 0.214 mg copper per oyster or from 8.21 to 13 77 mg per 100 g dry weight. In green colored oysters collected from adjacent areas only slightly contaminated with copper salts, the copper content in the flesh ranged from 1.27 to 2 46 mg per oyster or from 121.71 to 271 mg per 100 g dry weight (Galtsoff and Whipple, 1931; Galtsoff, 1964).

In a current study conducted at the Northeast Marine Health Sciences Laboratory, at Narragansett, R.I., Dr. B H. Pringle (unpublished data) found that the average copper content of oysters collected from unpolluted areas along the east shore ranged from 20 to 80 mg/l, wet weight; oysters from areas known to be polluted contained from 124 5 to 392 0 mg/l wet weight. The copper content of sea water ranged between 0 0038 to 0 005 mg/l in areas not known to be polluted. In certain polluted places, concentrations as high as 0 019 mg/l were recorded.

Other metals are easily absorbed, stored, and concentrated by oysters in great excess of their concentration in sea water Experimentally, it has been shown that iron and iodine can be absorbed within a relatively short time by oysters from water to which these metals have been added in excess The flavor of so-called superiodized oysters produced before World War II in Arcachon, France, was pronounced because the iodine content of flesh was many times higher than that in untreated oysters (Galtsoff, 1964) The color of the oysters was not affected

Green color of the gills of the European oyster in France and in the American oyster occasionally found in North Carolina and Chesapeake Bay is due to absorption of the blue-green pigment of the diatom, *Navicula,* present in large numbers on oyster grounds. The color is not associated with the increased copper content of flesh (Ranson, 1927).

Recommendation: To prevent the tainting of fish and other marine organisms, substances that produce tastes and off-flavors should not be present in concentrations above those shown to be acceptable by means of bioassays and taste panels Experience has shown that test organisms should be exposed to the materials under test for 2 weeks at selected concentrations to determine the maximum concentration that does not produce noticeable off-flavors as determined by organoleptic tests (Cooking should be done by baking the material wrapped in aluminum foil)

Plant Nutrients and Nuisance Organisms

Plant nutrients and nuisance organisms are interrelated in many ways There also are many other factors in the environment, such as temperature and salinity, that are closely correlated and, in many instances, seem to be contributing factors to nuisance organisms

Man, through altering the hydrography of his environment by building dams and diverting waterflows from their natural courses, has produced conditions in many areas that have caused nuisance growths and brought about an imbalance of natural conditions He also has enriched surface waters and created imbalances in dissolved materials and organisms through careless land management and by allowing the introduction of nutrients from sewers, food processing industries, fertilizer plants, feed lots, and farms. As a result, natural communities of aquatic life are altered and the functioning of these ecosystems often is changed severely or destroyed.

To maintain natural distribution, abundance, and interrelations of the aquatic biota, and to control unwanted growths, it is necessary to determine and maintain levels of dissolved materials required for this balance This is an extremely difficult task, however, because there are a great many interrelated factors that contribute to the development of excessive populations of a species. Although a considerable amount of work has been done on the nutrition of aquatic organisms, most of this work has been done on a very few different species. Very little research has been done to determine what interaction of factors causes a shift in diversity or in the kinds of species that compose a community. For these reasons it is impossible to set any definite requirements. At this time the only meaningful thing that can be done is to develop guidelines.

Plant Nutrients: The increase of nutrients in the sea is accelerated by deposition of material derived from the land as sediments from the rivers, by settling and filling caused by water movements produced by tide or wind, and by biological activity. To date, no serious problems resulting from abnormal enrichment of nutrients have been identified in the open sea except perhaps locally around outfalls that extend several miles out to sea. With the increased disposal of wastes in the sea, this potential problem should be carefully watched.

Estuaries and tidal embayments have long been recognized as some of our most valuable and productive resources They are the most ephemeral of the natural marine habitats and consequently most easily affected by man's activities. They serve as sinks for most of the organic and inorganic materials resulting from land erosion Because of the lack of scouring and the nature of the sediments that occur in some areas, anaerobic conditions often develop in the beds of estuaries and bays Increases in the deposition of suspended solids intensifies this condition. An excellent discussion of the role of sediments in an estuary is given by Carriker (1967).

Many industries and municipalities discharge nutrient-rich wastes into estuaries. Because of the nature of the estuary, these are recycled and accumulated over a period of time. Because of this recycling, effluents with low concentrations of nutrients may, in time, produce serious problems. The complete flushing of the estuary often takes many years With controlled water discharges, this problem may become more severe.

Plant nutrients consist of many types of chemicals For example, we have the chemicals commonly recognized as being important in plant nutrition such as nitrate, phosphate, sulfate, carbonate, calcium, magnesium, sodium, and potassium. There are also the so-called "trace elements" which are equally important but are required in small amounts such as iron, manganese, molybdenum, cobalt, zinc, etc. More recently the importance of organic compounds in plant nutrition has been recognized These include vitamins, such as vitamin B_{12}, organic forms of nitrogen, such as urea, various amino acids, and amides, and the simple sugars, such as glucose.

The role of dissolved organic compounds in the nutrition of plants as well as animals appears to be important Darnell (1967) refers to the aquatic medium as a "vegetable soup" to indicate its richness in dissolved organic materials The work of Ryther (1954) points out that the organic forms of nitrogen are best utilized by the less desirable species (*Nannachloris atomus* and *Stichoccus sp.*). *Nitzschia*, a desirable diatom, often grows poorly in their presence This is no doubt a major reason why sewage effluents often bring about the development of undesirable species.

If the increased nutrients in a system are well balanced, many species will have larger populations, the predator pressure will increase, and the productivity of the whole ecosystem will increase. If, however, the increased nutrients are of undesirable composition for most forms of aquatic life, or not in the correct ratio, excessive blooms of species with low predator pressures may develop. Examples of these are certain blue-green algae. Of course, environmental factors other than nutrients are important in the development of blooms. Any one important factor, such as temperature, light, or water mass stability, if limiting, may prevent blooms even though other conditions are suitable for their development. As a result, blooms sometimes do not develop even though most of the conditions are favorable.

Nuisance Organisms: Nuisance organisms in the marine environment are usually defined as those organisms which interfere with the use that man wishes to make of a particular water. Some examples are abnormally abundant growths of organisms that make bathing beaches unattractive, produce unpleasant odors, foul the bottoms of boats, spoil the esthetic appearance of water and the coastline, clog fishing nets, interfere with the flow of water within intake and effluent pipes, and interfere with navigation. This category of nuisance organisms should also include those organisms that interfere with the growth and reproduction of organisms important to man. For example, excessive populations of boring sponge or oyster drills, rooted and floating aquatics can interfere with the movement and reproduction of fish; bacteria and red tide organisms such as *Gymnodinium* and *Gonyaulax* may have toxic effects on other organisms, including man (Rounsefell and Nelson, 1966, Felsing, 1966).

The groups of organisms that may cause nuisances or become severe pests include algae (including red tide organisms), coelenterates, sponges, mollusks, such as oyster drills and mussels, and crustacea. These organisms are commonly encountered in the natural marine environment. Organisms may become nuisances because of excessive growth and changes in distribution patterns and predator-prey relationships. The main causative factors are excessive and, often, imbalanced nutrients, considerable changes in the natural regimes of temperature, turbidity, and salinity, and changes in current patterns

In some instances, nuisance growths seem to be directly related to the nutrients that are available. In other situations, nuisance growths may not be directly affected by artificial enrichment, so far as we know, and seem to be more strongly affected by changes in the temperature, salinity, or turbidity. Included here are various fouling organisms. barnacles, mussels and other mollusks, polyzoa tube worms, marine borers, and pests to useful marine products (oyster drills, boring sponges, crabs, parasitic fungi, and protozoans), and swarms of jellyfish, which make bathing in some coastal waters hazardous during certain seasons.

The effect of increased nutrients may be an increase in the populations of certain species already present in the environment and a decrease of species that are not tolerant of such nutrients. Examples of such conditions are the increase of *Enteromorpha* and sea lettuce, *Ulva lactuca,* in the zone of mineralization of sewage which occurs in some areas of the lower Potomac. In areas of higher salinity, abundant growths of *Ascophyllum* often occur in waters containing mineralized effluents from sewage treatment plants. In Biscayne Bay, Fla., the following organisms became abundant under such conditions· the flowering plants, *Halophila baillonis* and *Diplanthera wrightii,* and the echinoderm, *Amphioplus abditus.* Under heavy organic enrichment, the algae, *Gracilaria blodgettii* and *Agardhiella tenera,* the worm, *Diopatra cupera,* and the amphipods, *Erichthonius brasiliensis* and *Corophium acherusicum,* became very common (McNulty, 1955).

In other cases, an imbalanced organic enrichment together with changes in temperature and salinity brings about an almost complete change in the species composing an aquatic community plus excessive growths of some species. An excellent example of this type has been described by Ryther (1954) in his studies of Moriches Bay and Great South Bay, Long Island. In this area, duck farm wastes enrich the bay waters with organic compounds that produce a low nitrogen-to-phosphorus ratio At the times of the largest algal blooms, low salinities and high temperatures exist in the area. As a result, desirable marine diatom species of *Nitzschia* which prefer cool water (5 to 25 C), nitrates, and nitrites as a source of nitrogen, and are not benefited by a low N/P ratio (5:1) were replaced by *Nannochloris atomus* and *Stichococcus sp* These species can grow well in nitrates, nitrites, ammonia, urea, uric acid, and cystine, and prefer a N/P ratio of 5.1. As Ketchum (1967) points out, these weed species are undesirable food sources and the natural productivity of the estuary is destroyed Ketchum also points out that the greatest amount of plankton does not always occur in the waters of greatest enrichment. This is because the development of a maximum standing crop of phytoplankton is also governed by the concentration of predators, stability of the water column, transparency of the water, etc.

Nutrient imbalance may affect the ratio of inorganic phosphate to total phosphorus, here defined as the sum of inorganic, organic, and particulate phosphorus It is known from the work of Pomeroy (1960) and others that inorganic phosphorus is rapidly taken up by actively growing plants At the same time, inorganic phosphorus is regenerated as a result of bacterial degradation and excretion by animals. The net effect over the short run is to produce a steady state between the various fractions of phosphorus in the environment There should be some ratio of inorganic to total phosphorus in the euphotic zone that would be characteristic of a balanced nutrient regime and this ratio should be lower than the same ratio for the imbalanced system in which inorganic phosphorus can accumulate.

Data from Moriches Bay and Great South Bay on Long Island, Charlestown Pond, R.I , the North Atlantic, and the North Pacific have been examined In the obviously polluted portion of Moriches Bay, the inorganic total phosphate ratio generally exceeds 0 6, while the Charlestown Pond, an uncontaminated estuary of similar characteristics to Moriches Bay, this ratio was less than 0.4. In the open ocean at high latitudes and in the winter when phytoplankton density is low, the fraction of inorganic phosphorus may increase to 0 65 or thereabout.

Recommendation: The ecological factors most often associated with nuisance growths are changes in the natural temperature and salinity cycles and increases in nutrients The change in any of these factors may directly or indirectly affect the response of the organisms to other factors Increase or decrease in current and, indirectly, its effect on available nutritional materials have also been found to be important

To maintain a balance among nutrients and a balanced biota most conducive to the production of a desired crop, it is recommended that

(1) No changes should be made in the basin geometry, current structure, salinity, or temperature of the estuary without first studying the effects on aquatic life For example, these studies should be made before dams are erected, water diversion projects are constructed, or dredge and fill operations carried out

(2) The artificial enrichment of the marine environment from all sources should not cause any major quantitative or qualitative alteration in the flora Production of persistant blooms of phytoplankton, whether toxic or not, dense growths of attached algae or higher aquatics or any other sort of nuisance that can be directly attributed to nutrient excess or imbalance should be avoided Because these nutrients often are derived largely from drainage from land, special attention should be given to correct land management in a river basin and on the shores of a bay to prevent erosion

(3) The naturally occurring atomic ratio of NO_3-N to PO_4-P in a body of water should be maintained Similarly, the ratio of inorganic phosphorus (orthophosphate) to total phosphorus (the sum of inorganic phosphorus, dissolved organic phosphorus, and particulate phosphorus) should be maintained as it occurs naturally Imbalances have been shown to bring about a change in the natural diversity of the desirable organisms and to reduce productivity

Toxic Substances

Relatively few of the many substances recognized as potential toxic pollutants of the marine environment have been studied sufficiently to enable us to define their maximum allowable concentrations Specific pollutants and classes of pollutants are discussed in terms of current knowledge In some cases, data are adequate to set definite criteria, while in others, criteria are educated guesses at best and can serve only as temporary guidelines

Lethal concentrations of some persistent substances as determined by acute toxicity tests are so low that we are not justified in allowing their deliberate introduction into the natural environment. On the other hand, a few waste products appear to offer little threat to the marine environment because of their rapid degradation and dispersal.

Our concern is not primarily with what polluting substances are present, but whether or not they are present in sufficiently large amounts to cause deleterious effects on the biota and the environment. Many naturally occurring substances, including clean fresh water, would be toxic if discharged into the estuarine and coastal marine environment in sufficiently large amounts

Determination of the toxicity of known and unknown effluents, either simple or complex mixtures, can best be made by determining the reactions of endemic fauna exposed to them at levels that might be expected in receiving waters. Chemical assays may determine the presence of such pollutants at levels as low as nanograms per liter, but biological systems may be affected by even smaller amounts Many animals have the ability to accumulate toxic residues of substances present in the environment in only trace amounts until body residues are large enough to cause damage when released internally through normal metabolic processes Animals differ in their sensitivity to the same toxicant and it is essential that toxicity data be related, in the final analysis, to animals of economic importance

A fundamental concept in attacking the pollution problem is the assumption that effluents containing foreign materials are harmful and not permissible until laboratory tests have shown the reverse to be true It is the obligation of the agency producing the effluent to demonstrate that it is harmless rather than require pollution abatement agencies to demonstrate that the effluent is causing damage

Specific methods are suggested here for the determination of the toxicity of proposed effluents While certain procedures are desirable, they are not always reasonable and certain permissible alternatives are also given

Basic Bioassay Test: The basic bioassay test shall consist of a 96-hour exposure of an appropriate organism, in numbers adequate to assure statistical validity, to an array of concentrations of the substance, or mixture of substances, that will reveal the level of pollution that will cause (1) irreversible damage to 50 percent of the test organisms, and (2) the maximum concentration causing no apparent effect on the test organisms in 96 hours. Tests should be conducted, when possible, in a "flow-through" system so that the organisms are exposed continuously to a fresh solution of the test material appropriately diluted with water of the same quality as that at the site of the proposed discharge Adequate safeguards should be taken to insure that the test will be conducted under the least favorable environmental conditions that are allowable in the natural environment. Tests should be conducted at water temperatures typical of the mean of maximum daily temperatures during critical periods at the proposed effluent discharge site

Test organisms should be selected either on the basis of their economic importance in the area receiving the discharge and their sensitivity or on the basis of their importance in the food web of economically important animals. In the event that organisms meeting these criteria are not suitable or available for the confined conditions of the tests, substitute forms endemic to the area may be utilized Appropriate tests must be undertaken to demonstrate the relative sensitivity of economically important species and substitute species to the test material so that meaningful interpretations of the data can be made.

Application Factor: It is recognized that the most obviously deleterious effect of toxic substances is increased mortality. More subtle changes such as reduced growth, lowered fecundity, altered physiology, and induced abnormal behavior patterns may have more disastrous effects on the continued existence of the species. Evaluations of such sublethal effects generally will provide more meaningful guidelines.

It is recognized that there should be an application factor for each waste or material and that these factors may vary widely for these different wastes and materials The concept and use of application factors is defined and discussed at length in the toxicity portion of the section on water quality requirements for fresh water organisms. Due to a lack of knowledge of application factors for specific wastes and materials, a single application factor to be applied to all wastes is being suggested at this time. This application factor may require a lower concentration than is necessary in some instances, particularly for those materials that are subject to biological degradation, but it is known that it is not restrictive enough for some materials. Ideally, the determination of application factors should be the result of studies for the determination of safe levels of potential toxicants under long-term or continuous exposure The application factor is the concentration of a material or waste that is not harmful, divided by the 96-hour TL_m value for that material A few application factors have been so determined at the Bureau of Commercial Fisheries Laboratory at Gulf Breeze, Fla. (unpublished data). In the future, as application factors are determined for specific substances, they will replace the recommendation for the generalized application factor for these particular materials or wastes. It is clearly understood that as additional data become available recommendations on water quality requirements will be changed so that they conform with the new knowledge

Biological Magnification: Biological magnification is an additional chronic effect of toxic pollutants (such as heavy metals, pesticides, radionuclides, bacteria, and viruses) which must be recognized and examined before clearance can be given for the disposal of a waste product into natural waters Many animals, and especially shellfish such as the oyster, have the ability to remove from the environment and store in their tissues substances present at nontoxic levels in the surrounding water. This process may continue in the oyster or fish, for example, until the body burden of the toxicant reaches such levels that the animal's death would result if the pollutant were released into the bloodstream by physiological activity. This may occur, as in the case of chlorinated hydrocarbon pesticides (such as DDT and endrin) stored in fat depots, when the animals food supply is restricted and the body fat is mobilized The appearance of the toxicant in the bloodstream causes the death of the animal. Equally disastrous is the mobilization of body fat to form sex products which may contain sufficiently high levels of the pollutant so that normal development of the young is impossible.

The biological magnification and storage of toxic residues of polluting substances and microorganisms may have another serious after effect. Herbivorous and carnivorous fish at lower trophic stages may gradually build up DDT residues of 15 to 20 mg/l without apparent ill effect Carnivorous fish, mammals, and birds preying on these contaminated fish may be killed immediately or suffer irreparable damage because of the pesticide residue or infectious agent.

In the final analysis, laboratory tests alone are not sufficient to assess completely the toxic effects of a substance These data must be interpreted in combination with field observations Criteria established under the artificial conditions of laboratory tests will probably require adjustment in the light of later and more prolonged field observations

Recommendation: In the absence of toxicity data other than the 96-hour TL_m, an arbitrary application factor of 1/100 of this amount shall be used as the criterion of permissible levels

Additional chronic exposure tests will be conducted within a reasonable period to demonstrate that the estimated maximum safe levels as indicated by the

96-hour TL_m and the application factor do not, in fact, cause decreases in productivity of the test species during its life history

Monitoring the Marine Environment: The chief problem in monitoring the marine habitat for pollution lies in the fact that the discharge of toxic materials may be intermittent This is not necessarily true, but it means that water samples collected periodically reflect only the conditions at the time they were collected Significantly higher or lower levels of pollution may have existed between sample collections A second major factor for consideration is that trace amounts of pollutants or effluent mixtures toxic to the biota may not be readily susceptible to chemical analysis For these reasons, the analysis of resident biota for abnormal changes offers a better tool for interpreting environmental fluctuations

Mollusks are being collected for analysis at monthly intervals in estuaries on both the Atlantic and Pacific coasts (Butler, 1966 a, b). Analysis of resident populations by electron capture, gas chromatographic techniques reveal changes in residues of 11 of the more common organochloride pesticides which oysters, mussels, and some species of clams readily store. These methods are useful for rapid surveys of recent pollution. By appropriate spacing of samples in time and location, it has been possible to pinpoint sources of pollution.

It is suggested that a monitoring system of this type, appropriately expanded to include fish and plankton, would quickly identify areas where pollution problems exist Suitable analytical techniques are available to make these samples equally useful for the identification of pollution by heavy metals and other toxic substances

Monitoring for the presence of organophosphorous materials is feasible, but less specific for individual toxic compounds This group of pesticides exerts its toxic effect on living systems by inhibiting the enzyme acetylcholinesterase, which is essential to conduction in nerve fibers The nervous tissue of fish and some invertebrates, appropriately analyzed, reveals whether the organism has been exposed to organophosphorous materials within the past 2 to 4 weeks (Holland, et al., 1967) Identification of such changes can be made before toxicant levels are high enough to cause serious mortalities.

A particularly efficient nonspecific method for monitoring changes in the estuarine habitat is based on the periodic collection of sedentary animals and plants which have attached themselves to artificial cultch plates. Squares of asbestos cement boards placed in strategic locations will be utilized by resident biota as a habitat At 30-day or shorter intervals these plates can be changed, the organisms enumerated, volumetrically measured or chemically assayed, and an index of their relative abundance obtained (Butler, 1954).

Such plates have been maintained for nearly 20 years at one laboratory in Florida (Butler, 1965), and they supply detailed information on the relative productivity of the environment in relation to hydrologic changes. They will be equally useful as monitors of newly introduced pollutants in this area The monitoring method of choice—and there are others besides the ones suggested—will depend on the specific environment and the animals of particular interest No one method will be adequate and a combination of methods should provide the most information in the shortest time period.

Pesticides: Pesticides may be described as natural and synthetic materials used to control unwanted or noxious animals and plants. They exert their effect as contact or systemic poisons, as repellents, or in some cases as attractants. It is convenient to classify them according to their major usage such as fungicides, herbicides, insecticides, fumigants, and rodenticides. Although data are not available as to the total amount of pesticides used in the United States, total production figures (including exports) show that more than 875 million pounds were produced in 1965 This represents an increase of approximately 10 percent over 1964, and more than a fivefold increase in the past two decades In recent years, the use of herbicides has increased relatively more rapidly than that of other pesticides In 1964, more than 100 million acres of the continental United States were treated with some kind of pesticide. The trend in pesticide production is towards the manufacture of more granular formulations This physical adsorption of the pesticide on clay particles makes possible better control during application and should result in less dissipation of the chemical into atmosphere and into nontarget areas

Despite better control of pesticide applications, their dispersal in drainage systems and possible eventual accumulation in estuaries makes our coastal fisheries especially vulnerable to their toxic effects Estuarine oyster populations, juvenile shrimp, crab, and menhaden, for example, all occupy the habitat where fresh and salt water mix and where deposition of river silt with its load of adsorbed pollutants takes place Laboratory tests show that these economically important animals are especially sensitive to the toxic effects of low levels of pesticides. Oysters, for example, will exist in the presence of DDT at levels as high as 0 1 mg/l in the environment. But at levels 1,000 times less (0 1 μg/l), oyster growth or production would be only 20 percent of normal, shrimp populations would suffer a 20-percent mortality, and menhaden would suffer a disastrous mortality. Some insecticides are toxic enough to kill 50 percent or more of shrimp populations after 48 hours exposure to concentrations of only 30 to 50 nanograms per liter of the compound.

Pesticides may be classified by their chemical affinities and a large number of economically important insecticides are chlorinated hydrocarbon compounds. These include the well-known DDT and aldrin-toxaphene group Typically, these are persistent compounds, but they may be degraded by living systems into less toxic metabolites. As residues in soil and marine sediments, they may persist unchanged for many years and consequently present a continuing threat to animal communities. As a general rule, the acute toxicity of this group of pesticides increases with the level of metabolic activity so that their presence may cause two or three times more damage in summer than in winter months

The organophosphorous pesticides are also primarily insecticides Typically, they hydrolyze or break down into less toxic products much more readily than the organochloride compounds. Practically all persist for less than a year, while some last only a few days in the environment Most of them are degraded rather quickly in warm water and consequently are more hazardous to aquatic animals at winter rather than summer temperatures. They exhibit a wide range of toxicity, both more and less damaging to marine fauna than the organochlorides They are usually preferable as control agents because of their relatively short life.

Other major chemical categories including the carbamates, arsenicals, and 2,4-D and 2,4,5-T compounds are generally, but not necessarily, less toxic to marine biota

Pesticides registered for uses which might permit their dispersal into the marine environment must be evaluated for their toxic effect on oysters, fish, and shrimp Consequently, there is a considerable amount of information on the 48 or 96-hour TL_m values of these compounds Unfortunately, information is still lacking on their long-term effects at sublethal levels on the productivity of economically important marine species.

The extreme sensitivity of marine crustaceans, such as crabs, lobsters, and shrimp, to the array of insecticides is to be expected because of their phylogenetic relationship with terrestrial arthropods In general, shrimp are also much more sensitive than fish or oysters to the other pesticides This fact and their economic importance make shrimp a valuable yardstick for establishing safe levels of pesticides that might be expected as toxicants in the marine environment

A much broader spectrum of pesticide pollutants can be anticipated in the fresh water (salinity <0.5 ‰) zones of tidal estuaries. Fresh water criteria listed in another section will apply under these circumstances.

Recommendation: The pesticides are grouped according to their relative toxicity to shrimp, one of the most sensitive groups of marine organisms Criteria are based on the best estimates in the light of present knowledge and it is expected that acceptable levels of toxic materials may be changed as the result of future research.

Pesticide group A.—The following chemicals are acutely toxic at concentrations of 5 μg/l and less. On the assumption that 1/100 of this level represents a reasonable application factor, it is recommended that environmental levels of these substances not be permitted to rise above 50 nanograms/l. This criterion is so low that these pesticides could not be applied directly in or near the marine habitat without danger of causing damage The 48-hour TL_m is listed for each chemical in parts per billion (μg/l)

Organochloride pesticides

Aldrin	0 04	DDT	0.6
BHC	2 0	Dieldrin	0.3
Chlordane	2 0	Endosulfan	0.2
Endrin	0 2	Methoxychlor	4 0
Heptachlor	0 2	Perthane	3 0
Lindane	0 2	TDE	3.0
		Toxaphene	3.0

Organophosphorous pesticides

Coumaphos	2 0	Naled	3 0
Dursban	3.0	Parathion	1 0
Fenthion	0 03	Ronnel	5.0

Pesticide group B.—The following types of pesticide compounds generally are not acutely toxic at concentrations of 1 mg/l or less. It is recommended that an application factor of 1/100 be used and, in the absence of acute toxicity data that environmental levels of not more than 10 μg/l be permitted

Arsenicals	2,4,5-T compounds
Botanicals	Phthalic acid compounds.
Carbamates	Triazine compounds
2,4-D compounds	Substituted urea compounds

Other pesticides.—Acute toxicity data are available for approximately one hundred technical grade pesticides in general use not listed in the above groups These chemicals either are not likely to reach the marine environment, or, if used as directed by the registered label, probably would not occur at levels toxic to marine biota It is presumed that criteria established for these chemicals in fresh water will protect adequately the marine habitat It should be emphasized that no unlisted chemical should be discharged into the estuary or coastal water without preliminary biossay tests and the establishment of an adequate application factor

Heavy Metals: Heavy metal salts in solution may constitute a very serious form of pollution because they are stable compounds, not readily removed by oxidation, precipitation, or any other natural process. A characteristic feature of heavy metal pollution is its persistence in time as well as in space for years after the pollutional operations have ceased.

The number of substances that may be described as "poisonous" is very large and they vary enormously in the degree of their effect. For man and other air-breathing animals, the threshold dose of a toxic material generally means the maximum quantity that can be taken without causing death. For aquatic animals living in a water environment containing a toxic substance, the situation is somewhat different. Instead of receiving an absolute quantity at one time, they are being continually exposed to a given concentration of the toxic material. This is similar to a man regularly drinking water containing lead or breathing air containing a noxious gas or vapor. It is not surprising, therefore, that the student of pollution problems turns his attention toward the concentration of the poison he is investigating and the manner in which the effect is related to this, rather than to the absolute amount required to harm or kill. Animals have the ability to eliminate poisons at least to some degree or even to destroy them. Their ability to do this at a rate permitting survival depends on the concentration of the toxic material to which they are exposed.

One of the characteristics of living cells is their ability to take up elements from a solution against a concentration gradient. This is perhaps most obvious for marine organisms, especially for autotrophic algae which obtain all their nutrients directly from seawater. The ability of marine organisms to concentrate elements above that level found in their environment has been recognized for some time. The following points should be noted in relation to their concentrating ability.

(1) All elements are concentrated to a degree with the exception of chlorine, which is rejected, and sodium, which is weakly rejected. The concentration factors are of the order of one for bromine, fluorine, magnesium, sodium, and sulfur, and higher for all other elements.

(2) Among cations (including metallic elements such as iron, which may exist as colloids in the sea), the order of affinity for living matter is, generally: tetravalent and trivalent elements>divalent transition elements>divalent group II-A metals>univalent group I metals. The tetravalent and trivalent subgroup have rather different affinities for plankton and brown algae.

plankton: Fe>Al>Tl>Cr, Si>Ga
brown algae: Fe>La>Cr>Ga>Li>Al>Si

Similar differences are found between these organisms in their affinities for the divalent transition metals

plankton: Zn>Pb>Cu>Mn>Co>Ni>Cd
brown algae. Pb>Mn>Zn>Cu, Cd>Co>Ni

Of interest is the affinity of both organisms for lead, which has no known biological function.

It is clear that the heavier elements in these groups tend to be more readily taken up than the lighter ones, which may be connected with their greater, ease of polarization.

(3) The order of affinity of living matter for anions is

nitrate>trivalent anions>divalent anions>univalent anions

It is probable that most polyvalent metallic elements are more or less chelated by organic matter.

The main features of the uptake of ions by cells can be accounted for by assuming that another process operates apart from simple diffusion. This process is called active uptake and is closely linked with metabolic activities within the cell. The metabolic processes provide the energy necessary for the uptake against a concentration gradient. Active uptake has a larger temperature coefficient than does uptake by diffusion. In long-term experiments, the effect of temperature is probably complicated by increased rates of growth, cell division, and so on. Active uptake requires oxygen and occurs only in cells which are respiring freely. Substances which inhibit respiration also inhibit uptake of ions. The rate of uptake of ions may be limited either by the rate of exchange at the cell membrane or by bulk phase diffusion inside the cell. The former is usually limiting for ions present at low external concentration and the latter for ions at high external concentrations. It has been suggested that bulk phase diffusion limits the rates of uptake of most cations. There appears to be at least two active transport systems in addition to the diffusion processes. A large number of theories have been advanced to explain active transport. One of the most popular is the carrier hypothesis. Accordingly, the ions are transported across membranes as chelates with metabolically produced organic molecules.

Uptake by invertebrate animals.—The most primitive animals, the unicellular protozoa, take up ions from solution by diffusion in the same ways as do algae. Many marine species have vacuoles and these are able to open at intervals and extrude fluid from the cell. The vacuole regulates the osmotic pressure of the cell and thus controls its volume.

Multicellular invertebrate animals can be divided into two groups as far as uptake is concerned: those with permeable integuments and those without. The majority of marine invertebrates (colenterates, annelids, mollusks, and echinoderms) have soft bodies with permeable integuments through which ions can diffuse freely In this situation, the body fluid or blood is quite similar to sea water in composition The gills of mollusks are coated with a layer of complex carbohydrate sulfates which may function as ion exchangers The gills of marine Crustacea, which have hard impermeable carapaces, are fully permeable to water and salts.

Mode of toxic action.—An element is said to be toxic if it injures the growth or metabolism of an organism when supplied above a certain concentration. All elements are toxic at high concentrations and some are notorious poisons even at low concentrations For example, the essential micronutrient, copper, which is a necessary constituent of all organisms, is highly toxic at quite small concentrations The other essential micronutrients are also toxic when supplied in excess, though not all in such striking fashion There is an optimum range of concentration, which is sometimes quite narrow, for the supply of each element to each organism

When excessive amounts of an element are fed to an organism, they frequently cause death The usual measure of the amount required to cause death is called the LD_{50} This is the amount which, when fed to each individual in a population, kills half of the population. The LD_{50} is an imprecise measure unless it is qualified by specifying

(1) The chemical state of the element.
(2) The means of feeding
(3) The age or developmental stage of the organism
(4) The time elapsed between feeding and death

The most important mechanism of toxic action is thought to be the poisoning of enzyme systems. The more electronegative metals, notably copper, mercury, and silver, have a great affinity for amino, imino, and sulfhydryl groups which are doubtless reactive sites on many enzymes These metals are readily chelated by organic molecules We thus have discovered attempts to correlate metal toxicities with such factors as their electronegativities, the insolubility of their sulfides, or the order of stability of their chelated derivatives.

(1) Order of electronegativities of some divalent metals Hg>Cu>Sn>Pb>Ni>Co>Cd>Fe>Zn>Mn>Mg>Ca>Sr>Ba
(2) Order of stability products of the sulfides: Hg>Cu>Pb>Cd>Co>Ni>Zn>Fe>Mn>Sn>Mg>Ca
(3) Order of stability of chelates: Hg>Cu>Ni>Pb>Co>Zn>Cd>Fe>Mn>Mg>Ca

It appears likely that all the divalent transition metals, as well as the other electronegative metals, that form insoluble sulfides, such as Ag, Mo, Sb, Tl, and W, are poisons by virtue of their reactivity with proteins and especially with enzymes. In view of the large number of enzymes in living cells, the variations in toxicity indicated above are hardly surprising Studies have shown that metals giving rise to similar toxic effects may be acting on quite unrelated enzymes and also many more atoms of metal are absorbed by an inactivated enzyme than are required to block the reactive sites Other modes of toxic action are.

(1) Substances behaving as antimetabolites. This might be arsenate and chlorate occupying sites for phosphates and nitrates, respectively (Fluoride, borate, bromate, permanganate, antimonate, selenate, tellurate, tungstate, and beryllium.)
(2) Substances forming stable precipitates or chelates with essential metabolites. (Al, Be, Sc, Ti, Y, Zr, reacting with phosphate, Ba with sulfate, or Fe with ATP.)
(3) Substances catalyzing the decomposition of essential metabolites. (La and other lanthanide cations decompose ATP.)
(4) Substances combining with the cell membrane and affecting its permeability. (Au, Cd, Cu, Hg, Pb, U) These elements may affect transport of sodium, potassium, chlorine, or organic molecules across membranes or even rupture them.
(5) Substances replacing structurally or electrochemically important elements in the cell and then failing to function (Li replacing Na, Cs replacing K, or Br replacing Cl)

Metal-organic compounds may be either more toxic than the metal ion (ethyl mercuric chloride) or much less so (cupric ion and copper salicylaldoxime)

Silver.—Silver is present in seawater in a concentration of about 0.0003 mg/l It is found in marine algae at concentrations up to 0 25 mg/l and in marine mammals in the range of 1 to 3 mg/l (Vinogradov, 1953). It is highly toxic to plants and mammals.

Arsenic.—Arsenic is found to a small extent in nature in the elemental form. It occurs mostly in the form of arsenites of true metals or as pyrites

Its major commercial use is for pesticides (insects, weeds, fungi). Arsenic is cumulative in the tissues of many organisms and, therefore, it eventually exerts its effects even though the environmental level is low. It has been demonstrated to be a possible carcinogen in water.

Arsenic is found in seawater at a concentration of about 0 003 mg/l. It has been found in marine plants at concentrations up to 30 mg/l and is highest in the brown algae It is found in marine animals in a range of 0.005 to 0 3 mg/l. It is accumulated by coelenterates, some mollusks, and crustaceans (Vinogradov, 1953) It is moderately toxic to plants and highly toxic to animals especially as AsH_3.

Arsenic trioxide, which also is exceedingly toxic, was studied in concentrations of 1.96 to 40 mg/l and found to be harmful to fish or other aquatic life Work by the Washington Department of Fisheries (1944) on pink salmon has shown that at a level of 5 3 mg/l of As_2O_3 for 8 days was extremely harmful to this species Ellis (1937), using the same compound on mussels at a level of 16 mg/l, found it to be quite lethal in 3 to 16 days. Surber and Meehan (1931) carried out an extensive study on the toxicity of As_2O_3 to many different fish food organisms Their results indicated that important fish food organisms can tolerate an application rate of 2 mg/l of As_2O_3 The amount actually in the water is considerably less

Cadmium.—The elemental form of cadmium is insoluble in water. It occurs largely as the sulfide which is often an impurity in zinc ores

Cadmium is found in seawater at a level of less than 0 08 mg/l Its level in marine plants is approximately 0 4 mg/l, while in marine animals a range of 0 15 to 3 mg/l has been found. It is lowest in the calcareous tissues and is accumulated within the viscera of the mollusk, *Pecten novazetlandicae* (Brooks and Rumsby, 1965) Cadmium is moderately toxic to all organisms and it is a cumulative poison in mammals

Cadmium is used widely industrially to alloy with copper, lead, silver, aluminum, and nickel It is also used in electroplating, ceramics, pigmentation, photography, and nuclear reactors. Cadmium salts sometimes are used as insecticides and antihelminthics The chloride, nitrate, and sulfate of cadmium are highly soluble in water. The carbonate and hydroxide are insoluble, thus cadmium will be precipitated at high pH values.

Most quantitative data on the toxicity of cadmium are based on specific salts of the metal Expressed as cadmium, these data indicate that the acute lethal level for fish varies from about 0 01 to about 10 mg/l depending on the test animal, the type of water, temperature, and time of exposure. Cadmium acts synergistically with other substances to increase toxicity. Concentrations of 0.03 mg/l in combination with 0.15 mg/l zinc causes mortality of salmon fry (Hublou, et al., 1954).

Pringle (in press), in a study of adult American Eastern oysters, *Crassostrea virginica,* found an 8-week TL_m value of 0 2 mg/l of $Cd^{++}[Cd(NO_3)_2]$ and a 15-week TL_m value of 0 1 mg/l.

The most obvious effect, in addition to lethality, was lack of shell growth. A similar study on the clam, *Mercenaria mercenaria,* indicated that a much longer period of exposure at the same concentration was required to kill half of the test organisms.

Chromium.—Chromium is found in seawater at a concentration of 0.00005 mg/l. Marine plants contain approximately 1 0 mg/l while marine animals contain chromium within a range of 0.2 to 1 0 mg/l Chromium compounds may be present in wastes from many industrial processes or they may be discharged in chromium-treated cooling waters The toxicity of chromium varies with the species, temperature, pH, its valence, and synergistic or antagonistic effects (especially with hardness). Most evidence points to the fact that under long-term exposure the hexavalent form is no more toxic towards fish than the trivalent form. Doudoroff and Katz (1953), studied the effect of $K_2Cr_2O_7$ on mummichaugs and found that they tolerated a 200 mg/l level in sea water for over a week

The effects of hexavalent chromium on photosynthesis by the giant kelp, *Macrocystis pyrifera,* were as follows at 1 mg/l chromium, photosynthesis was not diminished by 2 days contact. It was reduced 10 to 20 percent by 5 days contact and 20 to 30 percent after 7 to 9 days The concentration of chromium required to cause a 50-percent inactivation of photosynthesis in 4 days was estimated at 5 mg/l (Clendenning and North, 1958, 1960, North and Clendenning, 1958, 1959).

Haydu (unpublished data) studied oyster mortalities and his results point out the long-term effects of low concentrations of chromium, molybdenum, and nickel. The levels of all three metals were in the range of 10 to 12 μg/l over a 2-year period. In addition, his data indicated that there were seasonal variations The mortalities at these levels increased with an increase in temperature. Approximately 63 to 73 percent of the mortalities occurred in the period of May through July, perhaps due to increased physiological activity (increased feeding and higher pumping rates)

This study substantiates the available evidence indicating that as the environmental level of these metals increases, the ingestion-elimination balance is upset, causing accumulation to take place

Raymont and Shields (1964), in studies with the small prawn, *Leander squilla,* found a threshold level of a little less than 5 mg/l Cr Thus, at chromium concentrations ranging from 10 to 80 mg/l Cr, 100-percent mortality occurred in 1 week, at 5 mg/l Cr no deaths occurred in 1 week although a few animals died over the subsequent 21 days. Larger prawns of the same species appeared to be considerably more resistant to chromium poisoning. The threshold was about 10 mg/l Cr Raymont and Shields in additional experiments on the toxicity of chromium to crustaceans (the shore crab, *Carcinus maenas*), indicated that chromium concentrations above 50 mg/l (Na_2CrO_4) were definitely toxic for a period of exposure of 12 days At 60 mg/l Cr, 50-percent mortality occurred after 12 days. At 40 mg/l Cr, 9 percent died within 12 days, while at 20 mg/l, an 8-percent mortality was observed. In studies on the marine polychaete worm, *Nereis virens,* these same investigators working in the range of 2 to 10 mg/l Cr found that there was heavy mortality with all solutions in 2 to 3 weeks. The threshold of toxicity appears to be at about 1 0 mg/l Cr level

Pringle (in press), in experiments using a well-controlled, flow-through system and chromium concentrations of 0 1 and 0 2 mg/l ($Na_2Cr_2O_7$), showed the average weekly mortality to be approximately 1 percent over a 20-week period. This was about the same as that for the sea water controls.

Copper.—Copper is found in seawater at a level of 0 003 mg/l It is found in marine plants at about 11 mg/l, while marine animals are found to contain 4 to 50 mg/l It is accumulated by some sponges and is essential for the respiratory pigment in the blood of certain annelids, crustacea, and mollusks In excess, it is highly toxic to algae, seed plants, and to invertebrates and moderately toxic to mammals Copper is not considered to be a cumulative systemic poison like lead or mercury

The toxicity of copper to aquatic organisms varies significantly not only with the species but also with the physical and chemical characteristics of the water Copper acts synergistically with zinc, cadmium, and mercury, yet there is a sparing action with calcium.

Barnacles and related marine fouling organisms were killed in 2 hours by 10 to 30 mg/l copper Clarke (1947) showed that the mussel, *Mytilus edulis,* was killed in 12 hours by 0.55 mg/l. Lobsters transferred to tanks lined with copper after living in aluminum, stainless steel, and iron tanks for 2 months, died within 1 day Copper is concentrated by plankton from surrounding water in ratios of 1,000 to 5,000 or more (Krumholz and Foster, 1957)

Concentrations of copper above 0.1 to 0.5 mg/l were found to be toxic to oysters by Galtsoff (1932) The 96-hour TL_m for oysters was estimated at 1.9 mg/l (Fujiya, 1960). Oysters cultured in waters containing 0.13 to 0.5 mg/l accumulated copper in their tissues and became unfit as a food substance. Pringle (in press) found the soft clam, *Mya arenaria,* extremely sensitive to copper. At a concentration of 0 5 mg/l, 100-percent mortality took place in 3 days. Using a 0.2 mg/l concentration at 10 and 20 C, all clams died within 23 days at the lower temperature, while at the higher temperature all succumbed in 6 to 8 days When 0 1 mg/l Cu at 20 C was used, all animals died in 10 to 12 days. Raymont and Shields (1964) in studies with the marine polychaete worm *Nereis,* showed that a concentration of 1 5 mg/l Cu was lethal in 2 to 3 days, and concentrations exceeding 0.05 mg/l Cu were lethal in approximately 4 days.

Clendenning and North (1958, 1960) and North and Clendenning (1958, 1959) evaluated the effect of copper (from the chloride and sulfate salts) on the rate of photosynthesis of the giant kelp, *Macrocystis pyrifera* With 0.1 mg/l of copper, net photosynthesis was inhibited by 50 percent in 2 to 5 days and 70 percent in 7 to 9 days. Visible injury appeared in 10 days. Copper was slightly less toxic than mercury but more so than nickel, chromium, lead, or zinc Marvin, Lansford, and Wheeler (1961) found 0 05 mg/l Cu toxic to *Gymnodinium breve* (red tide organism).

Mercury.—Mercury is found in seawater at a level of 0 00003 mg/l It is found in marine plants at approximately 0.03 mg/l.

Irukayama (1966) reported on a mercurial pollution incident in Japan, which was first recognized in 1953 A severe neurological disorder resulted in the area of Minamata Bay as a result of eating fish and shellfish from these waters. Many species of animals including waterfowl were succumbing to the "disease" called Minamata disease. Clinical features were cerebellar ataxia, constriction of visual fields, and dysarthia Pathological findings were regressive changes in the cerebellum and cerebral cortices. Investigation through 1965 suggested that the main cause was the spent factory waste of the Kanose Factory upstream from the Minamata Bay area. Methyl mercury compounds, waste byproducts from the acetaldehyde synthesis process, were being discharged and concentrated especially in shellfish

Ukeles (1962) made a study of pure cultures of marine phytoplankton in the presence of toxicants. One of the toxic materials used was lignasan (ethyl mercury phosphate) a bactericide-fungicide. She found lignasan to be lethal to all species at 0 06 mg/l and 0.0006 was the highest level used not causing drastic inhibition of growth.

Clendenning and North (1960) and North and Clendenning (1958) found that 0.5 mg/l of mercury added as mercuric chloride caused a 50-percent inactivation of photosynthesis of the giant kelp, *Macrocystis pyrifera*, during a 4-day exposure A concentration of 0.1 mg/l caused a 15-percent decrease in photosynthesis in 1 day and complete inactivation in 4 days. Mercury was more toxic than copper, hexavalent chromium, zinc, nickel, or lead. For phytoplankton, the minimum lethal concentration of mercury salts has been reported to range from 0.9 to 60 mg/l of mercury (Hueper, 1960). The toxic effects of mercury salts are accentuated by the presence of trace amounts of copper (Corner and Sparrow, 1956).

Lead.—Lead is found as a local pollutant of rivers near mines and from the combustion of leaded gasolines. The lead concentration in sea-water is in the order of 0 00003 mg/l. It is found in marine plants at a level of approximately 8 4 mg/l. Residues in marine animals reach a concentration in the range of 0.5 mg/l. It is highest in calcareous tissue.

Wilder (1952) found that lobsters died within 20 days when kept in lead-lined tanks, while in steel-lined and other types of tanks, they survived for 60 days or longer.

North and Clendenning (1958) found that lead was less toxic to the giant kelp, *Macrocystis pyrifera*, than mercury, copper, hexavalent chromium, zinc, or nickel

Pringle (unpublished data), in studies on the effects of lead on the Eastern oyster, *Crassostrea virginica*, found a 12-week TL_m value of 0.5 mg/l and an 18-week TL_m value of 0.3 mg/l. Concentrations of 0 1 to 0 2 mg/l induced noticeable changes in mantle and gonadal tissue under 12 weeks of exposure.

Nickel.—Nickel is found in sea water in a concentration of about 0 0054 mg/l. Marine plants contain up to 3 mg/l and this may be higher in plankton. Marine animals contain levels in the range of 0.4025 mg/l. Nickel pollution is caused by industrial smoke and other wastes. It is very toxic to most plants but less so to animals Haydu (unpublished data), in long-term studies with oysters, found that a level of 0.121 mg/l nickel caused considerable mortality.

Zinc.—Zinc is found in sea water in a concentration of 0.01 mg/l. Marine plants may contain up to 150 mg/l of zinc. Marine animals contain zinc in the range of 6 to 1500 mg/l. It is accumulated by some species of coelenterates and mollusks Speer (1928) reports that very small amounts of zinc are dangerous to oysters.

Clendenning and North (1960) and North and Clendenning (1958) tested the effect of zinc sulfate on the giant kelp, *Macrocystis pyrifera*. Four-day exposure to 1.31 mg/l of zinc showed no appreciable effect on the rate of photosynthesis, but 10 mg/l caused a 50-percent inactivation of kelp.

Other Toxicants

Ammonia-ammonium compounds.—Ammonia is found in the discharge of many industrial wastes. It has been shown that at a level of 1.0 mg/l NH_3, the ability of hemoglobin to combine with oxygen is impaired and fish may suffocate. Evidence indicates that ammonia exerts a considerable toxic effect on all aquatic life within a range of less than 1.0 mg/l to 25 mg/l, depending on the pH and dissolved oxygen level present.

Cyanides.—Hydrocyanic acid or hydrogen cyanide and its salts, the cyanides, are important industrial chemicals The acid and its salts are extremely poisonous.

Hydrogen cyanide is largely dissociated at pH levels above 8 2 and its toxicity increases with a decrease in pH. The toxic action of cyanides increases rapidly with a rise in temperature.

Fish can recover from short exposure to concentrations of less than 1 0 mg/l (which seems to act as an anaesthetic) when removed to water free of cyanide. They appear to be able to convert cyanide to thiocyanate, an ion that is not inhibitory on the respiratory enzymes. Complex cyanides formed by the reaction of CN with zinc or cadmium are much more toxic. However, the reaction between CN and nickel produces a cyanide complex less toxic than the CN itself at high pH levels.

Sulfides.—Sulfides in water are a result of the natural processes of decomposition, sewage, and industrial wastes such as those from oil refineries, tanneries, pulp and paper mills, textile mills, chemical plants, and gas manufacturing facilities. Most toxicity data available are based on fresh water fish. Concentrations in the range of less than 1 0 mg/l to 25 0 mg/l are lethal in 1 to 3 days.

Fluorides.—Fluorides are present in varying amounts in the earth's crust They are used as insecticides as well as in water treatment and many other uses While normally not present in industrial wastes, they may be present in trace or higher concentrations due to spillage. Data in fresh water indicate that they are toxic to fish at concentrations higher than 1.5 mg/l.

Detergents and surfactants.—During the past twenty years, synthetic detergents have replaced a majority of the soap products. Concern about their importance in pollution was heightened by the visible evidence of their foaming in the Nation's waterways. Their toxicity to the aquatic fauna has been very extensively studied, but for the most part it is difficult to establish safe criteria because of the varying conditions of the tests. Relatively little bioassay work on their effects on marine biota has been published, but it is indicated that, unlike soap, detergents are more toxic in highly saline water than they would be in the fresh water areas of tidal estuaries (Eisler, 1965, Eisler and Derrel, 1966).

The 96-hour TL_m values of an ABS detergent to five species of marine fish ranged from 7 to 22 mg/l (Eisler, 1965). Marine kelp were more sensitive and photosynthesis was inhibited 50 percent after 96-hour exposures to about 1.0 mg/l

Pathogenic organisms.—Oysters, clams, and mussels have a demonstrated ability to accumulate microorganisms, including bacteria and viruses, from their aquatic environments and to serve as a vehicle for the transmission of these microorganisms to their consumers (U.S. DHEW, 1956, 1958, 1962, 1965a; Liu, et al , 1967).

Controls to prevent the transmission of disease through this route have been provided in the United States through the National Shellfish Sanitation Program (NSSP) administered by the Public Health Service, Department of Health, Education, and Welfare on the behalf of the interested State and Federal agencies and the shellfish industry (1965b). This program has established bacteriological quality standards for those waters from which shellfish are to be harvested for direct marketing These standards, as described in the NSSP Manual of Operation, should be observed for those estuarine areas used for commercial production of shellfish for direct marketing (U.S. DHEW, 1965) The standards that are applied to shellfish harvesting areas have been revised periodically through the mechanism of a shellfish sanitation workshop held at 2 or 3-year intervals. As these standards are revised so should the water quality criteria be modified

Tar, gas, and coke wastes.—The distillation of coal for the production of gas, coke, and tarry materials used in the manufacture of dyes and various organic chemicals results in a watery waste known as ammoniacal gas liquor, the disposal of which can cause detrimental effects. Ammoniacal gas liquor contains free ammonia, ammonium salts, cyanide, sulfide, thiocyanate, and a variety of aromatic compounds including pyridine, phenols, cresols, xylenols, and aromatic acids After treatment to remove ammonia, the waste is called "spent gas liquor." Phenol or carboxylic acid is the most abundant of its many phenolic substances, probably the most dangerous to fish.

Phenolic substances are also present in materials used in road surfacing, sheep dips, and many industrial wastes such as those associated with the manufacture of plastics, dyes, and disinfectants. Gas liquor, discharged untreated to a stream, has an extremely high oxygen demand, many times greater than that of sewage. These various groups of organic substances produce a variety of effects on fish varying from intoxication and anaesthesia to paralysis and death.

Pure compounds representative of these groups found in such coal tar wastes have been shown to be toxic in ranges of 2 to 75 mg/l for cresols and 0 1 to 50 mg/l for phenols, for fresh water fish and lower aquatic life.

Petroleum refining and petrochemical wastes.—The volatile components of petroleum consist mainly of aliphatic hydrocarbons. In addition to paraffins and olefins, some petroleums contain relatively high percentages of naphthenes and aromatic hydrocarbons. The less volatile fractions of petroleum are used as fuels, lubricants, and construction materials (asphalt). These substances are somewhat more irritating to the skin and some are carcinogenic, but less so than coal tar products

Pulp and paper manufacturing wastes.—The types of pulp produced and pulping technology have undergone considerable change in the last 20 years and the trend continues. Modern pulpmills are geared to produce a variety of pulp grades due to the increasing demands for specialty products The characteristics of the waste waters from these specialty pulp grades can vary considerably. An example of this can be seen in the BOD loadings of the following sulfite grade pulps produced in a west coast mill:

Paper making—130 lb BOD/ADT (air dry ton).

Alpha hardwood—300 lb/ADT.

FAC-SAC—450 lb/ADT.

The major pulping processes include kraft, sulfite, semichemical, and nonchemical such as groundwood. The kraft process accounts for approximately 75 percent of the total pulp production in the United States. The number of mills using the sulfite process are declining, some are being converted to the kraft process.

From the standpoint of water pollution, kraft and sulfite mills are of great significance. The principal problems associated with pulpmill wastes are toxicity, depressed DO's, and slime growths. Clearcut cases of acute toxicity attributable to pulpmill wastes in modern times seldom exist except when spills or other accidents occur. It is much more common to encounter problems related to slime growths, depressed DO's, and to long-term or chronic effects on the biota.

A substantial portion of pulpmill wastes including the toxic components are very amenable to microbial degradation. In one study, kraft mill wastes were found to be nontoxic to oysters at a dilution of 1:20 when the BOD of the waste was reduced by 80 percent employing biological treatment In a similar study, the toxicity of kraft wastes to silver salmon was found to diminish proportionally to the degree of BOD reduction above 50 percent, again using biological treatment The results of a recent study by scientists of the International Pacific Salmon Commission indicate a fairly close relationship between BOD reduction and decrease in the toxicity of kraft wastes They found no apparent toxicity to salmon when the BOD was reduced by 65 percent While similar studies have not been made with sulfite liquors, there is some evidence that the toxic components of this waste are also degradable It is important to recognize that the biological mechanism or degradation involved in secondary treatment is essentially similar to that in receiving waters Given sufficient time, the process of degradation of the toxic components of pulp wastes also take place in receiving waters.

Because of the great complexity and variability of pulpmill wastes, it is difficult to find a satisfactory expression for concentration. Attempts have been made to relate toxicity to BOD, COD, total solids, PBI (Pearl Benson Index—a measure of the lignin content of pulp wastes), and various reference animals There is a general relationship with all of these criteria, i e., the higher the values, the greater the toxicity. Pulpmill dosages or dilutions have been used in bioassays on the basis of applied initial BOD. The response of the test animals has been found to vary considerably to given concentrations of applied BOD even from the wastes of the same mill. This would indicate that the concentration of toxicants in the total biologically amenable fraction is subject to considerable variation. This would not only explain the lack of a good relationship between the toxicity and initial BOD, but it would also explain why, on the other hand, there can coexist a good relationship between BOD reduction and reduction in toxicity. The latter is subject to degradation regardless of the proportions of toxicants and the other to biodegradable substances

The shortcomings of BOD as an expression of the concentration of toxicity would seem to be equally applicable to the PBI test. This test has been recommended as a measure of SWL (sulfite waste liquor) concentration. It measures the lignins in SWL which constitute an appropriate substance for tracing in receiving waters and for analysis due to their stability and high concentrations As indicated earlier, critical tests to determine the relationship between BOD reduction and reduction in toxicity have not been conducted with SWL. Nevertheless, there is sufficient evidence to indicate that the toxic components of SWL also reside in the biodegradable fraction and are also degradable The composition of SWL in receiving waters at different distances from the point of discharge would therefore differ even though similar PBI values may occur. The toxicity of fresh SWL at a PBI concentration of 50 mg/l would be much greater than of biologically stabilized SWL at the same PBI concentration. There is clear indication that further study of SWL toxicity and biodegradation is necessary

The toxicity of kraft and sulfite wastes to aquatic life is amply reported in the literature Deleterious effects produced by SWL (generally considered less toxic than kraft wastes) are reported from PBI values as low as 2.0 mg/l for oyster larvae to concentrations greater than 1,000 mg/l for the adult clams *Mya* and *Macoma*. Long-term bioassays with Pacific and Kumamoto oysters, carried out at Oregon State University using calcium-base SWL (10 percent solids), showed no adverse effects at 50 mg/l after 266 days of exposure Slightly deleterious effects were noted at 100 mg/l, indicating maximum safe limits lie between 50 to 100 mg/l Continuing field studies at Grays Harbor, Wash , support these findings. In bioassays conducted in salt water by the Washington State Department of Fisheries, salmon exposed for 30 days to concentrations of approximately 500 mg/l of 10-percent SWL showed no apparent ill effects Herring eggs, on the other hand, were adversely affected at concentrations greater than 96 mg/l

The apparent tolerance level for salmon in salt water using kraft wastes was found by the above investigation to range from dilutions of 1:16 to 1 90 after 14 to 30 days of exposure Growth studies conducted at Oregon State University by the National Council for Stream Improvement using raw kraft wastes in fresh water showed no adverse effects to salmonid fishes after 3 to 5 weeks exposure in dilutions of 1:100. English (in press), in his field studies of the English sole in Puget Sound, reports a sustained and thriving fishery in an area affected by SWL. Recent work by the Federal Water Pollution Control Administration (USDI 1967a) in Puget Sound showed damage to oyster larvae and developing English sole eggs at concentrations greater than 10 mg/l of 10-percent SWL. According to this report, oyster growth and market condition is adversely affected and phytoplankton productivity is inhibited at SWL concentrations over 50 mg/l.

Determining the toxicity of complex wastes like oil, refinery petrochemicals, and pulpmill wastes presents a number of problems. For one thing, they contain many known and, perhaps, equally as many unknown toxic substances in small quantities. The toxicological and other physical and chemical characteristics can vary considerably during any given day, in any given plant, due to changes in processes, sources of supplies, and the end product being produced. Considerable variation in effluent characteristics can occur even in a 1-day period. The resulting wastes from these industries contain upwards of several hundreds of compounds representing a number of homologous series of compounds from different organic groups. This complexity is augmented by the treatment of the wastes, as well as by the spectrum of products manufactured from the complex starting material used The relative ability to react biochemically and to exert an oxygen demand is characteristic of organic materials of such primary significance.

Many groups or series of compounds indicated to be present in such wastes have been shown to be toxic in varying degrees to aquatic life It is extremely difficult at this time, however, to place a concentration limit or set threshold criteria for such complex systems and hence should be individually bioassayed and their discharge managed accordingly

Waterfront and boating activities.—Increasing activities by commercial, military, and recreational vessel operators raise the specter of introduction of toxic materials in quantities sufficient to affect marine organisms adversely. This is particularly likely in the case of confined waters of small tidal tributaries, lagoons, embayments, and other marine areas employed as harbors.

Toxic materials are used to prevent activities of boring and fouling marine organisms Usually, however, every effort is bent in the case of toxic coatings to prevent rapid release of toxic materials into the environment since rapid loss reduces effectiveness of such coatings and increases costs. Some leaching is unavoidable—even necessary. Thus, the presence in confined harbors of many vessels whose bottoms are coated with toxic materials already presents hazards in some places This would be especially true after spring "fitting out" for small boats

Boatowners, boat and boatyard operators, fishing and commercial pier and marina operators are not especially noted for the care extended to nearby waters Commonly, everything that can be is flushed or jettisoned into the water. Purposeful discharges are many—though perhaps decreasing as emphasis on water pollution has increased. Paint leaching, paint spillage, oil and gasoline spillage, detergents, wood preservatives, exhausted containers, metallic objects of all types (zinc, copper, brass, iron, etc.), and other jetsam contribute to contamination from these sources.

Except for confined areas where there are many of these operations such as large shipyards, major military and commercial anchorages, and large and small boat anchorages, it is doubtful that toxicity from these operations is of serious proportions in tidal waters at this point. As with other fouling or contaminating activities of society, however, efforts should be made to keep biological damage from these sources to a minimum Some discharges are controllable and should fall under the same rules as industrial or community discharges. In the case of large marinas, shipyards, or major anchorages, requirements suggested elsewhere may have to be applied Future research should include specific attention to this aspect.

Similar comments can be made about waterfront structures and port operations There is considerable use of toxic materials in preservation of wood, steel, and masonry structures used on marine waterfronts Discharge of toxic materials, surfactants, petroleum products, other materials and jetsam is common Similar recommendations can be made for control and research as those for boat, boatyard, and vessel operations.

Disposal of laboratory wastes.—The rapid growth of marine sciences during the past decade is reflected in an ever-increasing number of stations and laboratories engaged in the study of various aspects of oceanography These institutions are located along the entire coastline of the United States: 28 on the Atlantic, 12 on the Gulf, and 29 on the Pacific About 2,500 persons (investigators, students, technicians, and laboratory assistants) are employed in these 66 establishments (Hiatt, 1963)

The above number includes institutions operated by Federal and State governments, by universities or privately endowed concerns which receive their main support from the government and national foundations. Other laboratories, hospitals, and research institutions operated by industrial concerns for their specific needs are not included in this total The laboratories range from small establishments, with less than four investigators, to very large institutions employing or providing research space for 200 to 500 investigators.

The types of research cover various fields of biology, microbiology, experimental physiology, biochemistry, chemistry, biophysics, molecular biology, radiobiology, fishery biology, fishery management, and industrial research. Consequently, the effluents discharged into estuarine and coastal waters vary from ordinary household sewage to mixtures containing an array of organic and inorganic compounds, drugs, and radioactive isotopes. The composition of these effluents cannot be predicted with certainty because the type of research varies greatly from year to year. The laboratory effluent is separated usually from the sea water system, which as a rule has independent plumbing, but is mixed with the domestic sewage and frequently is discharged into natural waters. When many scientific establishments are concentrated in a relatively small area, the situation may become serious. Chlorinated raw sewage entering the harbor a short distance from shore may be caught by a tidal eddy and for several hours circulate close to the sea water intakes of several laboratories before it is carried out by tides

To maintain desired water quality requirements for aquatic life, it is necessary to separate laboratory effluents from domestic sewage and provide treatment that renders them harmless to aquatic biota Under no conditions should highly toxic chemical compounds or drugs be permitted to be discharged into natural waters if toxic concentrations of them can be detected by chemical and physical methods.

Many marine laboratories are utilizing exotic and endemic microorganisms, some pathogenic, in research Extreme caution must be exercised to prevent contamination of water by introduction of biological materials which can harm marine organisms

Laboratory administrators should be responsible for the periodical examination of the toxicity of the effluent discharged into natural waters by their institutions

Recommendation: (1) Allowable concentrations of metals, ammonia, cyanides, and sulfides should be determined by the use of 96-hour TL_m values and appropriate application factors Preferably, the TL_m values should be determined by flow-through bioassays in which environmental factors are maintained at levels under which these materials are most toxic Tests should utilize the most sensitive life stage of species of ecological or economic importance in the area. Tentatively, it is suggested that application factors should be 1/100 for pesticides and metals, 1/20 for ammonia, 1/10 for cyanide, and 1/20 for sulfides.

(2) There is evidence that fluorides are accumulative in organisms It is tentatively suggested that allowable levels should not exceed those for drinking water

(3) The further dilution of wastes in marine waters suggests that the adoption of criteria established for detergents and surfactants in fresh water also will protect adequately biota in the marine environment.

(4) Bacteriological criteria of estuarine waters utilized for shellfish cultivation and harvesting should conform with the standards as described in the National Shellfish Sanitation Program Manual of Operation These standards provide that·

(a) Examinations shall be conducted in accordance with the American Public Health Association recommended procedures for the examination of sea water and shellfish

(b) There shall be no direct discharges of untreated sewage.

(c) Samples of water for bacteriological examination to be collected under those conditions of time and tide which produce maximum concentration of bacteria.

(d) The coliform median MPN of the water does not exceed 70/100 ml, and not more than 10 percent of the samples ordinarily exceed an MPN of 230/100 ml for a 5-tube decimal dilution test (or 330/100 ml where the 3-tube decimal dilution test is used) in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions

(e) The reliability of nearby waste treatment plants shall be considered in the approval of areas for direct harvesting

(5) It is also essential to monitor continuously waste from tar, gas, and coke, petroleum refinery, petrochemical, and pulp and paper mill operations. They all produce complex wastes of great variability, not only from facility to facility, but also from day to day This should be done on an individual basis with bioassays These tests should be made at frequent intervals to determine TL_m values as described for other wastes. For the more persistant toxicants, an application factor of 1/100 should be used while for unstable or biodegradable materials an application of 1/20 is tentatively suggested

(6) Concentration of other materials with noncumulative toxic effects should not exceed 1/10 of the 96-hour TL_m value For toxicants with cumulative effects, the concentrations should not exceed 1/20 and 1/100 for the above respective values

When two or more toxic materials that have additive effects are present at the same time in the receiving water, some reduction in the permissible concentrations as derived from bioassays on individual substances is necessary The amount of reduction required is a function of both the number of toxic materials present and their concentrations with respect to the derived permissible concentration An appropriate means of assuring that the combined amounts of the several substances do not exceed a permissible combination for the mixture is through the use of following relationship

$$\left(\frac{C_a}{L_a}+\frac{C_b}{L_b} \cdots +\frac{C_n}{L_n}\leq 1\right)$$

Where C_a, C_b . C_n are the measured concentrations of the several toxic materials in the water and L_a, L_b L_n are the respective permissible concentrations (limits) derived for the materials on an individual basis Should the sum of the several fractions exceed one, then a local restriction on the concentration of one or more of the substances is necessary

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FISH, OTHER AQUATIC LIFE, AND WILDLIFE

I INTRODUCTION

Research aimed at improving and maintaining the environment of aquatic organisms is not limited in objective to the benefit of these organisms. It is meant to provide adequate supplies of aquatic life and wildlife for man's use Thus, in approaching this problem, the question should be, "What is the quality and quantity of aquatic resources that man now has and should have in the future, and how can these resources be restored, protected, maintained, and enhanced?"

To meet these problems, the determination of water quality requirements for aquatic life is of outstanding importance. If a fresh water is suitable and safe for aquatic organisms, it can be rendered safe for human consumption by water treatment methods now in existence. If fresh water is satisfactory for aquatic plants and aquatic organisms, it will in most cases be satisfactory for livestock, wildlife, and irrigating crops. If a water is satisfactory for aquatic life, it will in general be aesthetically pleasing and suitable for most recreational uses. Water suitable for aquatic life should be entirely suitable for industry, navigation, and power. The establishment of water quality requirements for the protection of aquatic life resources is the key to the protection of most uses made of water.

In the past, only a small percentage of the available funds for controlling water pollution was utilized for the determination of water quality requirements for aquatic life and wildlife. Biological investigations were not carried out in proportion to their importance. Very little attention was given to determining environmental requirements of aquatic organisms and the concentrations of potential toxicants that are not harmful in the aquatic environment. As a result, the volume of work was entirely inadequate to meet the problem. Because of deficiencies in equipment and personnel, studies were short term and dealt largely with acute toxicity. Little research was done on determining the long-term effects of pollution, or levels of potential toxicants that are not harmful in the aquatic environment.

It was obvious to the Committee from the onset of their task that present knowledge is inadequate for establishing definitive and conclusive water quality requirements for aquatic life and wildlife. This deficiency became more obvious as the preparation of the Subcommittee Report on Water Quality Criteria progressed and the present consensus is that the elucidation of water quality requirements has barely begun. Only research that is broad in scope, pertinently oriented, carefully designed, and adequately financed will supply the information essential for the protection of fish, other aquatic life, and wildlife.

II RESEARCH APPROACHES, METHODS, AND PROCEDURES

Short and long-term bioassays are presently the only tools available for determining the toxicity of particular pollutants to aquatic life, other approaches are needed to provide an understanding of the mechanisms involved. The basic need is methods to study the effects of the environment on the organism. These analytical methods should provide a synthesis with predictive value for assessing the response of a given species to a new situation. The theoretical basis for such an approach is not yet worked out. A beginning can be made, however, by describing the relationships between environmental factors such as temperature, salinity, and the concentration of

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respiratory gases and the ability of an organism to succeed. Measuring the performance of the active and standard metabolic rates in key organisms with multifactorial combinations of various components in the environment would provide a valuable base line for determining the effects of pollutants. Research units should be organized to study the environmental physiology of aquatic organisms. Goals of the units would be to determine metabolic requirements of activity under a given set of environmental conditions and to understand the mechanisms which make such performance possible.

Parallel studies should also be made on the effects of environmental changes on the structure of the aquatic community (kinds, distribution, and abundance of species), food pathways, and energy flow. It is essential that we learn how environmental changes affect ecosystems, how ecosystems react to toxicants, under what conditions a new desirable ecosystem may develop, and what types of stresses initiate a chain of reactions which are extremely difficult, if not impossible, to stop.

A Selection of Test Organisms

The environmental criteria essential to aquatic life resources are difficult to determine. Extended, intensified, and sophisticated research is required to understand and manage the physical, chemical, and biological environment of fish, other aquatic life, and wildlife. The identification of environments which are (1) acutely harmful, (2) deleterious under chronic exposure conditions, or (3) safe under conditions of continuous exposure are very involved problems requiring research into the indefinite future.

These problems are compounded by (1) the large number of species involved, (2) the wide range of climatic conditions encountered in the United States and its territories, (3) the inherent differences among marine, estuarine, and freshwater environments, and (4) rapid industrial progress with the evolution of new contaminants, both real and potential.

These problems are so numerous that "short-cut" methods for finding answers must be developed. A desirable "short-cut" is limiting the total number of species of organisms to be studied, without creating serious gaps in the knowledge required. This should be possible because, in a given faunistic region, the number of abundant species which are important are relatively few in comparison to the total number in the area. It is recommended that test organisms be selected from those species of major economic and ecologic importance which can be held and reared successfully in the laboratory.

Even with this reduction, the number of species to be studied would be great and perhaps beyond the resources available for long-term studies. To help alleviate this problem, it is suggested that short-term sensitivity studies be done to determine which species and life stages are most sensitive to each environmental parameter or potential toxicant. Subsequent long-term studies to determine safe levels could be limited to the most sensitive species and life stages. The Committee believes that if the most sensitive species and life stages are protected, the entire biota will in most instances be protected. In some cases, it may be desirable to associate these studies on a priority basis with known wastes such as sulfite waste liquor or pesticides.

All the different faunistic areas of the Nation must be studied. These include Alaska, Hawaii, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, and American Samoa, as well as the various river basins, and the principal coastal zones.

Studies should be made to determine (1) the most important species in a particular biota, (2) methods for maintaining these species and their various stages in the laboratory so that they may be available in numbers sufficient for bioassays, and (3) the relative sensitivity of the various test organisms and life stages to the environmental factors and toxicant under investigation. By limiting sensitivity studies to those species peculiar to a given faunistic area, the total number under study could be limited, but collectively all of the nationally important species would be represented. When such an arrangement is operative, the species and life stages that are the most sensitive to each of the materials or wastes under study can be determined in a routine manner.

B Methodology

Even with the simplified outline described above, the task of determining safe levels remains prohibitive from the standpoint of time and cost unless short-term evaluations can be correlated with specific long-term changes or effects. It is, therefore, essential to devise and test several biological parameters which may furnish rapid indexes of environmental requirements and safe concentrations of toxicants.

In developing short-term methods, it is recommended that the procedures used in human toxicology be evaluated for applicability. Tests should include behavioral, physiological, biochemical, enzymatic, endocrine, metabolic, histological, histochemical, histopathological, and various blood studies when they appear necessary.

Current methods of bioassay indicate the upper levels at which a potential toxicant is intolerable. Short-term bioassays as now developed and used, do not indicate whether an environmental factor is at an optimum level or if it is deficient. It is recognized that short-term bioassays are inadequate for many purposes and that flow-through and long-term bioassays are needed. Many substances which act as environmental poisons depress energy utilization. Studies of energy utilization should permit determining when substances are relatively innocuous as well as when and to what degree they slow metabolism. It is proposed, therefore, that new approaches to water quality be sought. By using the energy concept, it may be possible to express water quality criteria in a more adequate fashion and research to accomplish this goal should have priority. Some of the suggested short-term methods for determining long-term effects may be effective in reaching these goals.

Another gross type of index may relate oxygen utilization and nitrogen excretion (the O_2 N_2 ratio) to biochemical imbalances and metabolic involvements. The oxygen-nitrogen ratio may possibly indicate the substrate being used for energy although it may not distinguish between fat and carbohydrates. Combined with CO_2 measurements, the ratios may offer a reasonable insight into the probable nature of substrates utilized. Abnormal oxygen-nitrogen ratios might result from certain types of sublethal metabolic anomalies that are caused by exposure to some toxicants.

Due to the magnitude of research needs for establishing meaningful water quality requirements for fish and other aquatic life, major emphasis will have to be placed on laboratory bioassays and on developing new and better bioassay techniques to meet existing problems. Increased efforts, therefore, must be expended to make bioassays more realistically reflect situations that occur in the field under natural conditions. Properly conducted experiments for comparing laboratory results with field observations will provide a sound scientific basis for establishing correct criteria with respect to temperature, oxygen, and other environmental requirements.

Methods must be developed for detecting subtle changes which indicate slow deterioration in the aquatic environment. These studies are essential if methods are to be developed for field use, because slow deterioration is the most difficult effect to detect and prevent. Subtle environmental conditions or low concentrations of potential toxicants must be studied. While these do not cause death or any easily-observed harmful effects, they will diminish growth and productivity, decrease the general well-being, or even in time destroy the population or eliminate a species. This is a problem similar to the one faced in the protection of man from the effects of air and water pollution. Death is obvious, but the gradual lowering of the general health, well-being, productive capacity, and slow deterioration of physical ability are not so obvious. There must be research to develop methods to detect, measure, evaluate, and control the subtle effects of pollution so that aquatic life resources on which we depend for food, recreation, and environmental pleasures can be restored and maintained.

III RESEARCH NEEDED

A Chemical Investigations

In the past, chemical analyses made in connection with water pollution surveys and investigations have not been specifically directed toward toxicological problems. Analyses were confined to those materials for which analytical methods had already been developed. Usually, these tests were not sensitive or accurate enough to detect and quantitate materials at the low concentrations at which they are chronically toxic or safe. Also, tests were not designed to quantitate materials in their toxic forms. For example, total cyanide is measured as CN ion. This is not only misleading but inappropriate in a toxicological sense. Not all cyanides are equally toxic and when they are altered to and measured as CN ions, the result is that more cyanide is reported than actually occurs in one or several toxic forms. Experimental evidence indicates that it is the undissociated HCN molecule which is taken up by the organism. Thus, methods must be developed to detect and measure undissociated HCN in mixtures of complex cyanides. This is essential for the meaningful toxicological evaluation of cyanide wastes. The same is true for the H_2S molecule, the un-ionized NH_3 and the NH_4OH molecule. Research on analytical methods for the development of micro-analytical toxicological procedures are essential for a sound water quality program. Specific analytical methods must be developed for all potential toxicants so that they can be measured in water and tissues at the very low concentrations at which they are chronically toxic. At present, only a few analytical procedures are available while several others have not yet been refined to meet the speed and sensitivity needed in toxicological work.

Research is needed in the field of biochemistry for the detection and quantitation of enzymes, metabolic products, hormones and changes resulting from exposure to toxic materials. Special emphasis must be placed on developing analytical methods of the specificity, sensitivity, and accuracy essential in toxicological evaluations.

Chemicals or wastes that affect the different functions or organs should be evaluated, i.e., materials or families of products which affect respiration, digestion, reproduction and, on the molecular basis, the enzymatic systems involved in these functions. The particular physicochemical changes tested should be those induced by materials introduced in the environment and known to produce definite stresses on the organism. These materials should be studied under both laboratory and field conditions. Furthermore, methods are needed to determine the effect of two or more biologically active materials that act either in unison, antagonistically, or synergistically. The causes and mechanisms of synergism and antagonism should be examined. Knowledge of the basic governing principles may serve in forecasting consequences that result from introducing specific products or wastes.

Methods should be developed employing radioactive tracers to aid in determining toxicant-altered metabolic pathways, the metabolism of toxicants themselves, and their turnover time or concentration sites. Chemical and automated physicochemical techniques should be adapted to complement ecological investigations.

B *Environmental Requirements of Aquatic Organisms*

Information is needed on the essential and optimal environmental requirements of aquatic organisms. While some work has been done on temperature effects and oxygen requirements, it has been limited to a few species that are adaptable to laboratory conditions. Precisely controlled and monitored research must be carried out with the important and most sensitive species of each of the major classes of organisms to determine favorable and allowable variations of environmental factors.

Some work has been done on short-term, acute lethal changes but the effects of long-term exposures must be evaluated. Data on the adaptive processes associated with the sublethal changes are essential in determining environmental conditions favorable for the well-being and the production of a crop of aquatic organisms. Studies are needed on the interactions of these environmental factors and the levels at which various combinations of these factors are most favorable. Research needed on some environmental factors will be listed and discussed in turn.

1 Dissolved materials

Included under this heading are those materials which are not usually considered as potential toxicants because they are only toxic when present in abnormally high concentrations. The most common materials falling within this category are the salts of the earth metals. Studies should be made to determine for aquatic life the most favorable levels of these materials alone and in association with other materials, as well as the most favorable relative concentrations.

2 Turbidity, settleable and suspended solids

Settleable and suspended solids and turbidity are of great importance in the aquatic environment. Any particle suspended in water absorbs light and decreases transparency. The effect of attenuating light cannot, however, be separated from physical, chemical, and biological effects of suspended materials on the aquatic biota. Only the decrease in the rate of photosynthesis can readily be linked to increased turbidity. However, sedentary organisms, their eggs, and larvae may be affected by the settling out of excessive amounts of silt, clay, detritus, and other materials suspended in the water. Plankton, expecially the diatoms, may be covered with sediment and lose their buoyancy, thus settleable materials may effectively reduce the plankton population by settling out. The gills of fishes and bivalves can be injured by sediments, therefore, the maximum concentrations which are not harmful to important species should be determined.

Research is needed on the following·

a The acute and chronic effects of various concentrations of presumably inert substances, such as calcareous silt, sand, kaolin and others can adversely affect the respiration, feeding, reproduction, growth and behavior of important aquatic species. Examples of these species are bivalves, crabs, shrimp, lobsters, fishes of commercial and recreational importance, and their food organisms.

b Behavioral effects of silting and sedimentation on the aquatic biota under simulated natural conditions where variables are controlled.

c Physiological, chemical, and biological effects of surface phenomena associated with suspended particles, their affinities for different chemicals, and their use as substrates for microorganisms.

d The determination of the qualitative and quantitative penetration of different kinds of light in relation to the kinds and sizes of suspended materials.

e The determination of the light requirements of plants, both quantitatively and qualitatively.

f Development of methods for determining the physiological effects on fishes and aquatic invertebrates of long exposure to high turbidities. Laboratory studies are needed to determine tolerable levels of turbidity or suspended materials for different groups of aquatic organisms such as phytoplankton and zooplankton, molluscs, aquatic insects (especially the Plecoptera, Emphemeroptera, Trichoptera, and Diptera) and effects on their productivity.

g Laboratory studies to determine the effects of turbidity on migration of organisms and its influence on flow-drift of organisms.

The effects of settling materials on the bottom have been studied to some extent, but much more information is needed on the direct and indirect effects of turbidity on species available for food or recreation. Laboratory and field studies are needed to determine the effects of sediments on aquatic organisms and the amounts of these materials which are detrimental. Studies are needed to indicate specific effects of these materials and measures or indexes of tolerable limits.

3 Color and transparency

Research is needed to determine the long-term biological effects of 25, 50 and 100 units of color derived from different sources on the growth and reproduction of phytoplankton, zooplankton, benthic organisms, and submersed plants. Information is needed on the extent to which a particular color affects the amount of light received at a given depth. Studies should be made on the biological consequences of color independent of the direct biochemical effects of the products inducing the color.

4 Salinity

Emphasis should be placed on the physico-chemical properties of seawater. Research is needed to determine the tolerable and favorable ranges of salinity for important organisms and the variable toxicity of pollutants at various levels of salinity.

There is still much to be learned about the physiology and behavior of estuarine animals or amphibiotic organisms migrating through estuaries. How do they regulate their internal environment under the radical changes of salinity? What are the energy requirements under different combinations of the varying characteristics of estuaries and how do the organisms meet these requirements? How do pollutants affect animal resistance when combined with euryhaline migrations? How can the animal survive the added burdens offered by pollution?

5 pH

Some of the general effects of pH changes on other environmental requirements and on the toxicity of materials and wastes are known, but much remains to be learned concerning the pH ranges which are tolerable and favorable for a large number of species. Data are needed on the specific requirements of the various organisms and the limits--upper and lower--of pH which affect them adversely or are lethal. More study is needed on the effects of pH on toxicity, oxygen requirements, tolerable carbon dioxide levels, and the availability and utilization of nutrients.

6 Temperature

Problems of temperature and dissolved oxygen are interrelated and should be considered as interdependent variables. Research on the following points is urgently needed. Do daily fluctuations in temperature have any biological significance? How rapid can the change be in either direction? Does it matter if the fluctuations are out of phase with the natural cycle? What are the relative effects of wide fluctuations above a daily mean? How much variation can incubating eggs withstand?

Information is also needed on the effects of seasonal fluctuations. Are seasonal fluctuations essential? How important are they in triggering spawning? Are they required for successful incubation of the eggs? Do they affect availability of food organisms? There is need to know how much of a temperature shock fish and other important aquatic organisms can withstand if swept into plumes of heated effluents, or if fish, especially amhibiotic forms, avoid areas of unfavorably high temperature. There is need to determine the transition in temperature that migrating fish can tolerate.

Another outstanding problem is that of the selective favoring of species by changes in temperatures and thus altering or nullifying an endemic balance of species. There is need to know the levels of temperature which are tolerable and favorable for all important species in the aquatic environment, marine, estuarine, and freshwater. At what temperatures will desired species be replaced by undesirable competitors? As temperatures increase, what are the trade-offs relative to increased growth and total productivity versus parasites, disease, shifts in species composition, and overall mortality? Information is required on the levels of temperatures which are tolerable and favorable for all important species in the different aquatic environments. Data are required on the specific effects of high, but sublethal temperatures, the duration of elevated temperatures which may become lethal, those which are detrimental and those which favor optimal development. Studies should be carried out to determine the ability of organisms to withstand long periods of high sublethal temperatures which extend beyond the normal summer period. Data are needed on the low temperature requirements of important organisms and the effects of these low temperatures on the development of sex products and the completion of the normal life cycle.

Studies are needed to determine if the acute or chronic effects of sublethal, high temperatures become more harmful when compounded with stress, such as toxicants, starvation or salinity changes. Information is urgently needed on the temperature requirements for spawning, egg, and larval development of all species important for recreational, commercial, and forage purposes. Analysis of the behavioral and physiological mechanisms resulting in a particular performance should reveal some of the basic effects of temperature. Thermal tolerance diagrams are also needed for a larger number of the economically important freshwater and anadromous fishes. Temperature-selection curves and temperature-activity curves are required for most if not all important species.

7 Oxygen

While a considerable number of studies have been carried out to determine the minimum dissolved oxygen concentrations required by certain species, a great deal of research is needed to determine the dissolved oxygen concentrations tolerable and favorable for all important species. The effects of low as well as high oxygen concentrations should be investigated. The effects of various continued levels of dissolved oxygen on growth rate, as well as the effects of periodically induced variations in oxygen content, and short-term exposures to low or high concentrations of dissolved oxygen should be determined. The metabolic rates measured as oxygen consumption by certain important species under various conditions of temperature and salinity of the water should also be evaluated. Tests should be conducted under controlled conditions, in properly designed and constructed metabolic chambers. It is desirable that each test be conducted for significant periods of time to obtain accurate levels of basal metabolism. Carbon dioxide produced during the test should be measured to obtain the respiratory quotient values (RQ). Effects of toxicants on metabolic rates, oxygen requirements and RQ should be studied using the important species for which controlled measurements have been previously obtained. Studies are needed to determine the oxygen requirements for the fertilization and development of eggs, development and growth of larval and juvenile forms as well as the effects of dissolved oxygen on the activity and reproductive cycle of the adults.

8 Carbon dioxide

Information is needed on the levels of carbon dioxide which are tolerated for short or extended periods by various organisms and levels which do not produce unfavorable effects under conditions of long-term exposure. Information is needed on the photosynthetic needs for carbon dioxide. Buffer systems that are able to insure desired productivity should be developed. Buffering systems essential to insure desired productivity should be developed to promote desired growth. Extensive research is needed for determining the concentration of nutrients which produce undesirable blooms.

9 Nutrients and nuisance growths

Some of the factors contributing to excessive growth are known, however, concentrations and relative proportions of fertilizing material and other environmental factors essential to the production of undesirable blooms are unknown. Laboratory experiments can help define the conditions which promote these blooms in nature. In carrying out such studies, experiments of graded complexity can proceed from controlled laboratory experiments towards the sum of variables and the near duplication of the natural environment. Eventually, experiments should be carried out in controlled sections of natural streams, or in artificial streams, lakes, reservoirs, and estuaries to duplicate natural conditions. In this way, it would be possible to study specific chemical and physical factors as they affect particular species living in a complex ecosystem, as well as the effects of such factors on predatory relationships. Questions to be answered by research are, to name a few·

a How are nutrient balances affected by the addition of given chemicals, under the influence of various day lengths, pH, temperatures, etc.?

b How do shifts in nutrient balance affect the abundance of diatoms, blue-green algae, green algae and rooted aquatics?

c How do these shifts affect the abundance of species which have high or low food values?

d What species are the preferred food for the important organisms in the ecosystem?

e Is a diversified diet of many species (diatoms and some green algae species) a better source of nutrition than a single species source?

f As the human population and its organic waste continue to increase, how can nutrients be balanced or treated to promote desirable growths rather than nuisance blooms?

g What are the best methods for removing nutrients from water and for the control of eutrophication?

h How can mineralized wastes and sludges be used productively?

i What kinds of bacteria and fungi are important in assimilating wastes in rivers and what environmental factors stimulate their development and the effective conversion of wastes to harmless materials?

j How can the heat discharged in an effluent be used with beneficial effects or with minimized harmful effects?

k What are the degrees of interdependence among species of groups? Studies on the effects of limiting species diversity could aid in this.

l Which are the most important organisms in the food web?

m Is energy more efficiently transferred through a high or low diversity of species within a food web?

n How can our rivers be managed so that the shallow water areas support desirable aquatic organisms?

C Toxic and Damaging Materials

1 General

Largely due to the activities of man, many toxic materials are already present in the aquatic environment. The number is increasing continually due to the proliferation of new materials and industrial development. There already exists a great backlog of materials for which the acute toxicity to aquatic organisms should be determined. An even greater task is the determination of the long-term effects of these materials. While short-term bioassays have indicated the acute toxicity of several wastes to a few aquatic organisms, there is little information on concentrations of these materials which are chronically harmless to aquatic organisms of recreational, economic, and forage importance. Safe levels of these materials must be determined for all important sensitive species if the biota is to be protected. Information is lacking also on the possible antagonistic or synergistic effects of combinations of these materials on selected species.

2 Tastes and odors

There are a number of materials which may, at sublethal concentrations, taint fish flesh, or produce odors and tastes which are undesirable. Studies are needed to determine those materials and organisms that produce tastes and odors in water supplies, as well as methods for their elimination on control. Studies should be carried out to determine materials that taint flesh of aquatic organisms, the length of time required for tainting, the maximum concentrations of these tainting substances which do not produce tastes or odors, and the time needed to render the tainted organisms for use.

3 Oils

Studies should be conducted at sites of oil pollution to determine

a Chemical composition of oil slicks at their point of origin and at increasing distances from it and the composition of oil sludges and their change in relation to time and distance from the spillage source.

b Accumulation of water soluble toxic compounds in oil polluted waters, identification of them, and determination of their toxicity.

c Rate of degradation of oil under controlled laboratory conditions and in the natural environment with special emphasis on the effects of oxidation on oil toxicity.

d Gas formation in oil sludges, its identification and the carrying capacity of gas bubbles in moving or transporting oil to the surface to form new oil slicks.

e Interaction of various water soluble fractions of oil with the organic compounds naturally present in the water.

Studies should also be made to determine the following

a The survival of adults, larvae, and eggs of selected species in different concentrations of various grades of crude oil, fuel oil and kerosene.

b Effects of oil accumulation on the gas exchange apparatus of gill-breathing organisms. Aquatic insects, oysters, soft-shell clams, bay scallops, flounders, etc. are of interest here.

The following problems should be investigated in the coming years.

a Anatomical injuries to fishes and other aquatic animals resulting from explosives used for oil exploration and other purposes.

b Effects of explosions on behavior of individual animals, school formation, and stock migration.

c Effects of vibrations on behavior of individual fish and other animals and on populations of important species.

d Effects of vibrations on spawning, fertilization of eggs, and egg development.

e Effects of explosions and vibrations on plankton.

4 Highly toxic wastes

Most of the studies on the toxicity of various materials to aquatic organisms have been short-term bioassays with pure compounds. While a few studies on complex wastes have been made, they have been limited due to the nature of the tests and the inability to secure large amounts of these wastes. Recently, a number of long-term studies have been made where low concentrations of toxicants have been maintained over the entire life cycle of a test organism. These relatively few observations have indicated that the application factor to be applied to TL_m values to denote safe concentrations during continuous exposure may range from 1/7 to 1/500 of the 96-hour TL_m. Application factors recommended by the National Technical Advisory Subcommittee on Water Quality Requirements for Fish, Other Aquatic Life and Wildlife are 1/10, 1/20, and 1/100 of the 96-hour TL_m. It was realized that in certain instances these factors would be either over-protective or possibly inadequate. Newly-determined application factors should replace these interim values as rapidly as scientific observations are completed. Research to determine application factors for the most important wastes is imperative.

The following research projects involving studies on highly toxic wastes, should be given priority for adequate protection of aquatic life.

a Development of methods for raising all life stages of test organisms in order to complete essential laboratory bioassay studies.

b Short-term bioassay studies to identify the most sensitive species and the most sensitive life stage to selected materials and wastes.

c Long-term bioassay studies with those species and life stages which are the most sensitive to a particular waste to determine the following

1) The consequential effects of non-lethal damage at any stage on successive stages in the life history.

2) The effects on all life stages of sublethal exposures during the most sensitive stage.

3) Comparative studies of the effects of exposure during the most sensitive stages and of exposures during the entire life-cycle.

d Determination of the normal range of natural environmental fluctuations on the toxicity of various materials.

e Associated with investigation of the long-term effects of various toxicants, the following studies should be made.

1) Determination of the mode action, of toxicants, their metabolites, and detoxification mechanisms.

2) Determination of the interaction of two or more toxicants, synergistic and antagonistic effects, and those materials with similar and different modes of action.

f Studies should be initiated to develop short-term methods for determining long-term effects. These studies should include the following

1) Behavioral studies to detect any change that may render the organism unable to cope with the natural environment or make it susceptible to predation or disease.

2) Physiological studies to detect any adverse functional effects of exposure to sublethal concentrations of toxicants.

3) Investigations of effects on enzyme or endocrine gland functions.

4) Metabolic studies evaluating changes due to the action of toxic materials.

5) Blood studies for the development of methods that may indicate subtle sublethal effects.

6) Histological, histochemical, and histopathological studies to detect changes due to sublethal concentrations of toxicants.

7) Use and adoption of methods developed by medical science for the detection of toxicants and the evaluation of their effects.

g Bioassay experiments should be designed to permit adequate statistical analysis of the results. Reliable formulae for the computation of application factors could thus be derived.

h Bioassay methods are needed for arthropods, molluscs, worms, phytoplankton, zooplankton, and bacteria. Studies are needed for improvement and standardization of bioassay procedures, for the utilization, development, adoption, and improvement of bioassay instrumentation, for the monitoring and control of water quality and for the detection and the measurement of pollutants.

i Research is needed to determine the toxicity of degradable toxic products and to determine the mechanism and rate of their degradation.

5 Radioactive wastes

Research is needed for determining the following.

a Behavior and fate of radionuclides in the aquatic environment and their concentration by aquatic food organisms.

b The influence of the physicochemical state of the radionuclides and the characteristics of the dynamic equilibrium within the ecosystem on the availability of the radionuclides to the biota.

c The effects of environmental conditions upon the biological concentration of radionuclides and their passage through food chains.

d The damaging effects of the continuous exposure of sensitive stages of important aquatic organisms to radionuclides.

D Ecological Field Investigations

Although laboratory experiments can provide valuable information on how an organism reacts under controlled conditions, tney do not necessarily reveal how an organism will react under the stresses found within the complex ecosystems of natural waters. There are many instances where the predictive values of such laboratory experiments are poor This is probably due in great measure to the largely artificial environment in which they are carried out Therefore, some of these experiments should be extended to areas duplicating natural environments, such as pilot areas or areas in sections of natural waters which can be monitored or controlled for the purposes of these studies Ecological field studies are needed to evaluate laboratory findings for the favorable ranges of such environmental factors as temperature, dissolved oxygen, carbon dioxide, dissolved solids, suspended solids, settleable solids, turbidity, color, light requirements, currents, salinity, and pH.

1 Methods

Available field methods are not adequate to meet the problems which we now face in the detection and measurement of various environmental factors and their effects on the aquatic biota. There is a need to develop facilities, equipment, and methods for field testing so that necessary studies can be carried out under natural conditions for determining harmful or favorable effects. Better sampling methods are needed for both grab samples and continuous monitoring. More accurate methods for compositing samples and taking samples from composites should be developed. Better instrumentation is needed for continuous recording of environmental factors. Better methods or models are needed for relating the conditions in streams, lakes, reservoirs, estuaries, or coastal areas to various kinds of land use and to various kinds and amounts of effluents being discharged into these waters. To improve the evaluation of sublethal or obscure effects of potential toxicants, more precise field methods are needed for determining the effects of pollution on the ecosystem and for measuring standing crops and productivity at the various trophic levels. Autopsy techniques should be developed for determining the cause of kills, the concentrations of the toxicants or their metabolites in the organism, and the relationship of these compounds to lethal or sublethal effects.

2 Environmental requirements

a Temperature Field studies on the effects of heat are necessary under pilot plant conditions or in the environment where conditions are the same or as nearly approaching natural conditions as possible. Ecological observations should be conducted in the areas affected by discharges of heated water from power plants. Experiments conducted in tanks, troughs, or raceways under controlled conditions should be carried out to determine the survival of species studied. These studies should include effects of temperature and its variations on the competition of the different species, the effects of extremes in temperature and temperature variations on the movement of aquatic organisms, their spawning, egg development and survival, development of larvae, the presence of growth anomalies, and the production of desired species.

b Dissolved oxygen Field studies are needed to determine oxygen concentrations which are lethal, or unfavorable, and those which are favorable under natural conditions where there is competition for food and space, and where the other stresses found in a natural environment are present. Such studies are needed for fresh, estuarine, and marine waters. Much can be learned by field ecological studies of the occurrence and well being of populations of important species in waters with normal as well as different oxygen concentrations and different daily and seasonal variations.

c Toxic materials Special pilot study areas, controlled natural areas, or natural areas must be used for the field evaluation of laboratory findings on toxicity. Natural areas may also eventually be used for determining the effects of a toxic material or waste on the entire biota of a water.

The direct translation of laboratory data to the field situation is very difficult and often unsatisfactory. Under natural conditions, there may be a rapid reduction in the concentration of a toxicant by one or more of the following. precipitation, adsorption on soils and bottom materials; chemical decomposition, reactions with other substances in the water, absorption by microscopic organisms, removal by organisms and biochemical degradation. Accumulation of these materials in the food chain and ingestion of food organisms bearing relatively high concentrations of these materials may increase the exposure to the higher organisms.

Laboratory findings on the safe levels of potential toxicants and environmental requirements must be field tested under conditions where the organisms in question are exposed to all the stresses occurring in the natural environment. Such tests should be carried out before water quality requirements are recommended. When developed, tested, and evaluated, field studies can be used for simultaneously testing all the species in the biota under natural conditions. In such studies, biological magnification, storage, passage through the food chain, accumulation in bottom materials, competition for food, cover and living space, the effects of disease parasites and predators, synergism, antagonism, and the interaction of materials and all other complicating factors present in the natural environment are taken into consideration. When effective studies can be made in streams, lakes, reservoirs, estuaries, and coastal areas, the determination of safe levels of potential toxicants in the environment will be made in much less time. Such field studies will require development of improved methods for estimating the biota and seasonal changes in the biota, as well as development of analytical methods for continually monitoring the actual consideration of potential toxicants in the areas under test. Where known pollutants occur and fishery statistics are available, field studies properly designed and carried out can be the most convincing means of determining effects of such pollutants. A great deal can be learned by evaluating the effects of nature's bioassay, i.e., evaluating the biota that has developed and maintained itself under certain environmental conditions. Observations of populations and population dynamics, in different areas having different levels of wastes, can be very informative in indicating relative sensitivity and the overall effects upon the biota of the additions of these wastes.

III WILDLIFE

A The needs for wildlife are largely met if the requirements for aquatic life are satisfied. However, additional research is needed for the determination of special requirements essential to wildlife. The following research studies should be expanded to determine

1 Distribution of pesticides, heavy metals, and trace elements in the wildlife food chain and the tissues of various species, expecially migratory birds.

2 Analytical methods to differentiate between the compounds present in the environment versus the absolute amount of compounds available for absorption by plants and animals, e.g. Hg and Se.

3 The plants and animals that concentrate specific chemicals to levels capable of intoxicating other organisms in the food chain. Elements of toxic significance are antimony, arsenic, beryllium, bismuth, cadmium, chromium, lead, lithium, mercury, nickel, selenium, silver, tillurium, and thallium.

4 In addition, studies should be made on the effects of environmental nutrient elements present in excessive amounts or in deleterious combinations. Among these are barium, boron, calcium, cobalt, copper, iron, manganese, magnesium, molybdenum, potassium, sodium, and zinc.

5 New or better analytical methods for identification and quantitation of nutrients, toxicants, and trace elements, their movement through the food chain, their biological magnification, and where, when, and in what form they accumulate.

IV SUMMARY

The objective of this proposed research is to determine the water quality requirements of aquatic life and wildlife. To accomplish this objective there must be an effective, efficient, adequately supported, and continuing national research program. This program must be planned and carried out so that adequate data are secured to establish (1) maximum or minimum environmental conditions and concentration of toxicants which shall not be exceeded, (2) environmental conditions and concentration of toxicants which can be tolerated for short periods without significant harm, (3) the range of environmental conditions and concentrations of dissolved materials which are not harmful and those which are favorable and required for all important species.

A Research Approaches, Methods and Procedures

Research must be conducted to determine new or better methods for the following purposes.

1 To devise or modify and develop equipment and methods for the collection, isolation, and production of all life stages of important aquatic organisms so they are available at all times in sufficient numbers for bioassay studies.

2 To devise or modify and develop effective bioassay methods and other procedures for determining environmental conditions and concentrations of potential toxicants which should not be exceeded, which can be tolerated for short periods and which are favorable and essential for the survival, growth, reproduction, and general well being of all important groups of aquatic life and wildlife.

3 To develop facilities, equipment and ecological, analytical, physiological, toxicological, and other methods for the field and laboratory determination of the effects on the biota of alterations in environmental conditions and the presence of various concentrations of potential toxicants.

4 To develop special equipment or methods for determining or developing·

a The effects of the environment on the total biota and individual organisms to provide a synthesis with predictive values for indicating the responses of a species or biota to a given change in the environment.

b The active and standard metabolic rates under multifactorial combinations of various environmental components in the presence of pollutants.

c New and more specific methods for detecting sublethal effects of toxicants such as the oxygen-nitrogen ratio and bioenergetics.

d Methods for sampling and monitoring, instrumentation for automatic continuous sampling, more precise field methods for determining effects of pollution, and determination of standing crops, productivity, and population dynamics.

e Autopsy techniques

B Chemical Investigations

Research is needed for.

1 The development of microanalytical and other toxicological methods for the detection and quantitation of toxic materials in water and tissues at the extremely low concentrations at which they are either toxic or safe under conditions of continuous exposure.

2 The development of analytical methods of the specificity, sensitivity, and accuracy essential for toxicological studies.

3 Developing methods for using radioactive tracers to aid in determining metabolic pathways

of toxicants, their breakdown products, and their turnover time or concentration in tissues.

C Environmental Requirements of Aquatic Organisms

Research should be carried out to determine the environmental requirements of all stages of the life history of important sensitive species to indicate lethal levels or sublethal but harmful levels of environmental factors, and the ranges of these factors that are favorable and promote the production of a desirable crop. Research for these purposes will include the following studies.

1 Temperature and dissolved oxygen Laboratory and field research should be carried out to determine the following

a What is the significance of daily and seasonal fluctuations in temperature and dissolved oxygen?

b How rapid may changes be without exerting an adverse effect?

c Does it matter if the fluctuations are out of phase with the natural cycle?

d What are the effects of wide fluctuations above or below the daily mean and how much can temperatures be raised and dissolved oxygen concentrations lowered from natural conditions without harming the biota or causing a change in its makeup?

e At what temperatures and dissolved oxygen levels will desired species be replaced by undesirable competitors?

f What are the trade-offs relative to increased growth and total productivity versus parasites, disease, and mortality of different life stages?

g What levels of temperature and oxygen are harmful, tolerable, and favorable for all the important species at all life stages, including egg, nymph, larvae, fry, etc.?

h What are the effects of changes in daily and seasonal temperatures on migration, scope of activity, and the toxicity of waste materials?

i What are the maximum temperature and minimum oxygen levels that can be tolerated for short periods without harm?

j What are the allowable overall increases in temperature at different seasons that do not produce undesirable effects on the biota?

k What are the allowable and favorable levels of temperature and oxygen for all important species?

l What are the requirements for low temperatures in the life cycle of important organisms?

m What are the effects of stress due to pollutants on the temperatures which are lethal, tolerable, or favorable?

n What are the beneficial effects of the addition of heat?

2 Nutrients and eutrophication

a What are the nutrient requirements of desired species and the organisms in their food web?

b How much can nutrient conditions be changed from the normal without detrimental effects?

c What are the amounts of nutrient or fertilizing materials which may be desirable from the standpoint of productivity?

d How do changes in nutrient balance, due to additions of wastes or other materials, affect the abundance of different groups or species of organisms and the eutrophication of a water?

D Other Environmental Factors

Dissolved materials Determination of high or low concentrations which can be tolerated for short periods and the favorable range of concentrations or levels for the following. dissolved solids, suspended solids, settleable solids, turbidity, color, salinity, pH, and carbon dioxide.

E Toxic Wastes and Materials

The following research should be conducted in connection with the toxicological program.

1 Long-term studies with the most sensitive species and life stages of important organisms should be conducted. They would serve to determine the maximum concentration of potential toxicants which, under the extremes of allowable environmental conditions, are not harmful with long-term or continuous exposure as indicated by survival, growth, activity, reproduction, and the general well-being. Studies for determining long-term effects should include the following:

a Behavioral studies to detect any changes that may render the organism less capable to cope with the natural environment or make it more susceptible to predation or disease.

b Physiological studies to detect any adverse functional effects of exposure to sublethal concentrations of toxicants.

c Investigations of effects on enzymes or endocrine gland functions.

d Metabolic studies to evaluate changes due to the action of toxic materials.

e Histological, histochemical, and pathological studies.

2 Devise, develop, and test short-term methods for determining long-term effects of toxic materials on aquatic organisms through the use of metabolic, physiological, biochemical, behavioral, histological, histochemical, histopathological, enzymatic, hormonal, stress, energy transfer and balance, and other tests carried on in conjunction with long-term laboratory tests.

3 Studies to determine the following.

a The effects of non-lethal damage during any developmental stage on successive stages.

b The effects on all life stages of sublethal exposure during the most sensitive stage.

c Comparative studies of the effects of exposure during the most sensitive stages and the complete life cycle exposures.

d The mode of action of toxicants and mechanisms of detoxification.

e The interaction of two or more toxicants, synergistic and antagonistic effects, and different modes of action.

4 Determination of application factors for use with TL_{50} values to determine safe discharge rates for industrial wastes.

F Radioactive Waste

Studies should be conducted to determine allowable levels of radioactivity in the environment which are harmful to the aquatic biota or produce concentrations in the bodies of aquatic organisms which render them unsuitable for food.

G Tastes and Odors

Research to determine: (1) materials and organisms responsible for taste and odors in water supplies and the flesh of edible organisms, (2) the maximum concentrations of these materials which do not produce objectionable tastes or odors and methods for their control.

H Wildlife Requirements

Research to determine new or better analytical methods for identification and quantitation of nutrients, toxicants, and trace elements. Also, we need to know about their movement through the food chain, their biological magnification, and where, when, and in what form they accumulate. Other needed investigations include:

1 Studies on the sources, disposal, treatment and elimination of oils from the habitats of waterfowl.

2 Studies on the effects of pollutants or changes in water quality on waterfowl and wildlife food organisms.

3 Determination of concentration of herbicides (used for controlling nuisance plants) which do not produce long-term pathological effects or tainting.

4 Determination of the effects of chemical mosquito control on waterfowl and other organisms in the biota.

SECTION G

SOME CURRENT POLLUTION PROBLEMS

Water usage generally is followed by return of the used water to the source from which it was taken, or to some other body of water. Alteration of the characteristics of water typically results from usage. Wherever these alterations interfere with subsequent use of the water, they are regarded as pollution.

In this Section, a selection of outlines identifies typical changes in water as a result of current uses, and describes the general nature of the effects of such use on the water.

Land use, eutrophication of lakes and streams; thermal pollution; the phenomena of biological magnification (including mutagens, teratogens, and carcinogens), oil spills; siltation; and non-point sources are all current problems of environmental management greatly significant to man.

Contents of Section G

EFFECTS OF POLLUTION ON AQUATIC LIFE

I INTRODUCTION

A The effluent from any given industrial plant may be combined with municipal sewage, and/or wastes from other industries. This may occur in the sewerage system or in a natural body of water.

1 Toxic wastes may inhibit biota of treatment plant as well as life in receiving stream.

2 Organic wastes may simply increase sewage-type load on plant and stream.

3 The above effects may be reinforced or neutralized by a complex of industrial wastes.

B The general overall character of a body of water may be subtly changed over a period of time.

II The principle of limiting factors (see Figure 1) deals with the response of organisms to various factors in the environment.

A Liebig's Law of the Minimum (Figure 1) states that the distribution of a species may be limited by one or more essential environmental factors which occur in minimal quantities.

B Shelford on the other hand pointed out in his Law of Tolerance (Figure 1) that there are also maximum values of most environmental factors which can be tolerated. In between these two extremes there are ranges which may be called "optimum" for factors useful to the organism. Purely deleterious factors on the other hand have a maximum tolerable value, but no optimum. The range between the maximum concentration (greater than zero) which kills no organism and the minimum concentration which kills all organisms is known as the "critical range."

C These principles apply to all aquatic life whether in a stream, lake, estuary, or treatment plant. They are the basis for the control or regulation of biological conditions.

III INDIRECT TOXICITY: MODIFICATIONS OF THE ENVIRONMENT WHICH AFFECT AQUATIC LIFE

A Deposition of inert precipitates and silt tends to smother bottom organisms. Contributing materials include silt or sand from erosion due to poor agricultural practices, rock flour or tailings from mining or quarry operations, mica, coal washings, sawdust and debris from lumbering, insoluble precipitates or complexes from chemical industries.

1 Vulnerable organisms include important fish foods such as insect larvae and snails, also fish eggs, bottom-living algae such as diatoms, and many others.

2 Physical injury to delicate membranes of eyes, and gills may also result.

3 Inert suspended materials and dyes reduce light penetration, suppress photosynthesis and hence biological productivity. They also prevent game fish and other predators from seeing their prey, thereby reducing the efficiency of food utilization.

The word "stream" should be interpreted in most cases to mean "river," "lake," "estuary," etc., as applicable.

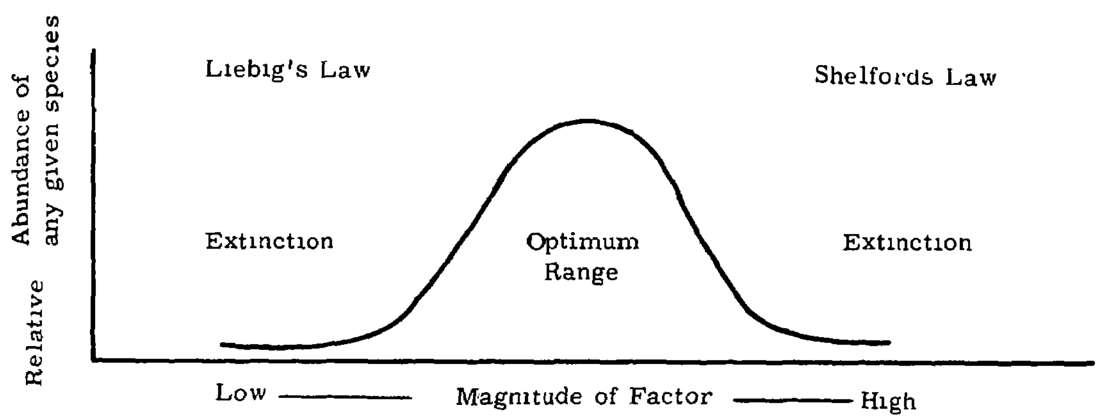

Figure 1. EFFECTS OF ENVIRONMENTAL FACTORS

B Wastes of significant heat content may change the "climate" of a body of water. Temperature may be higher or lower than normal.

1 Abnormally low summer temperatures may prevent the reproduction of some of the typical inhabitants, on the other hand colder water forms such as trout may be enabled to survive.

2 Abnormally high winter temperatures may encourage a rapid development of some species, thus for example, causing an early emergence of some insect followed by its death from normal winter temperatures. Southern forms may also invade more northerly waters, sometimes leading to a year-round nuisance from flying swarms of adults such as caddis flies.

3 Artificially produced high temperatures, often lack dependability. It is discouraging for organisms to spend six days in summer temperatures in January, only to freeze to death over the weekend because no one warned them that the plant would shut down!

4 Excessively high summer temperatures, even for a few hours once a year, probably represent the greatest temperature danger. Some species of fish can adjust to temperatures approaching 100° F, if the change takes place slowly. Each species however, has some maximum temperature above which it cannot survive (Figure 1).

a Quick temperature changes are fatal at much lower values.

b Lack of oxygen due to low solubility at high temperatures or from an increase in the rate of the BOD, also contributes greatly to high temperature mortalities.

C Oxygen-consuming wastes kill by depleting the free dissolved oxygen resources.

1 Amount present, rather than percentage of saturation, is usually more significant.

2 Minimum amounts rather than averages are most critical.

a Any species can survive something less than the optimum concentration of DO for a limited period of time. There is, however, some concentration for any given temperature, which will eventually result in the death of that species. Let us call this the "critical" DO.

b As the DO falls below the critical concentration, survival time eventually drops to zero.

c The absolute values of these thresholds vary with the species and other factors. Five mg O_2 per liter is often listed as a minimum permissible value to maintain a well-rounded healthy population of fishes on a year-round basis.

3 Low oxygen tensions may also increase the toxicity of certain chemicals.

D pH

1 "pH" is a logarithmic expression of the hydrogen ion concentration in a solution. Hydrogen ions (H+) in certain concentrations are toxic to aquatic life (as are also hydroxyl ions OH^-). Aquatic life in relative abundance and variety can be found in waters ranging from approximately pH 5 to 9. Thriving communities including algae, insects, and fish have been studied in waters with a pH of at least 11.

2 Many species of aquatic organisms can adjust to pH values over a wide range. Sudden change of any kind however can be fatal.

3 Most metals and other toxic substances in dilute solution tend to become less toxic at high pH values. A notable exception is ammonia.

IV POLLUTION WHICH RENDERS FISH OR SHELLFISH UNUSABLE OR INTERFERES WITH THEIR CAPTURE

A Radioactivity at levels currently found in our waters has not been observed to adversely affect aquatic life itself. It may however, be taken up with food materials and render fish or fishery products unusable.

1 Radioactive nuclides (forms of chemicals) are taken up by the plants (predominantly algae) in the processes of photosynthesis and other types of protoplasmic synthesis. There is no selection between nuclides on the basis of radioactivity.

2 As the chemicals originally assimilated by the algae are "eaten up the food chain" (from algae to invertebrates to small fish to large fish to man and other predators) their radioactivity moves along with them.

3 Thus radioactivity is acquired by fishes essentially through food, and scarcely at all by direct assimilation or absorption.

4 Plankton-feeding organisms such as herrings, oysters, and clams acquire radioactivity directly from the algae on which they feed. Since they concentrate this food from large volumes of water, they may be much "hotter" than the surrounding water itself.

B Fish may be repelled or driven out of an area by obnoxious chemicals. This may simply result in their scarcity or absence from a given locale, or it may prevent their swimming up a river to spawn. In this case the species would soon disappear and be lost to the community.

C Color, odor, oil, floating scum, bacterial slimes, and other such materials tend to discourage sport fishing and interfere with gear used by commercial fisheries.

D Sublethal concentrations of chemicals such as phenol, benzene,oil, 2-4-D, etc., may impart an unpleasant taste to fish flesh, even when present in very dilute concentrations. This is nearly as detrimental to the fisheries as a complete kill, and of course applies to shellfish as well as fin fish.

E Minamata disease was first described from Minamata, Japan, as a disorder resulting from eating various seafoods taken from Minamata Bay, Kyushu, Japan. The disease results from industrial toxicity, in this case an organic mercury compound, transmitted to a wide variety of local marine seafood species. These organisms are not known to be affected, but acting as "transvectors," pass the toxin along to predators or human consumers. Over 30% mortality occurred in Minamata among people eating local seafood.

1 It may be important to note that a fish kill was recently reported from a TVA lake in this country resulting from mercury leaching from corroded 50 gallon drums used as floats by marinas.

2 Bird and fish kills have recently occurred in Swedish lakes resulting from mercury compounds from pulp mills. Levels in pike exceed the WHO standards for human consumption and the local population has been advised not to eat pike more than once a week! Cases of high mercury levels are increasing.

V DIRECT TOXICITY: AFFECTS THE ORGANISM ITSELF

Fish kills are often the result of direct toxicity. If this is sufficiently potent to kill at once, or within a few days, it is called acute, and is often observed as a "fish kill."

Action that may require weeks or months to be effective may be referred to as low-level, cumulative, or chronic toxicity, and is more often observed as simply a reduction of productivity: "Fishin' ain't what it used to be." Examples of chemicals often believed to be involved include: acids, alkalines, ammonia, chlorine, cyanides, metals, phenols, solvents, sulfides, synthetic organic chemicals, oil field brines, pesticides, herbicides, detergents and others.

A Acute toxicity may be so broadly effective that many forms of life are affected at one time, or it may be highly selective. It may result from a low concentration of a highly toxic material or a high concentration of a relatively less toxic material.

1 It is frequently encountered as a "slug" resulting from a dump or spill, followed by normal, relatively non-toxic conditions as the mass of water containing the poison flows on downstream, or is deflected by tidal movements.

2 Acute toxicity can be evaluated by means of the toxicity bioassay technique and various modifications (Figure 2).

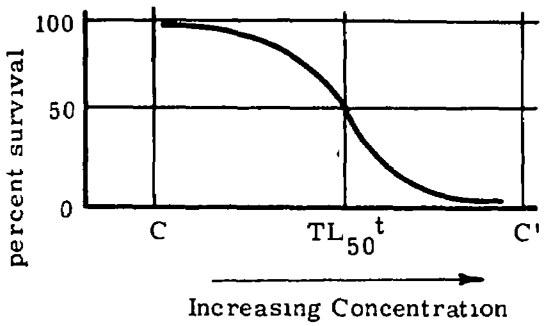

Figure 2 - Critical Range

C: maximum concentration at which no fish die, C': minimum concentration at which all die. TL_{50}^{t}, 50% tolerance limit (concentration tolerable to 50% of the population) for time t.

B Chronic or low-level toxicity may change the entire population balance.

1 Susceptible species of either fish or fish food organisms may die off, thereby permitting tolerant species to flourish for lack of competition.

2 If algae and/or invertebrate food organisms are killed off fish may die or move out of the area.

3 Weakened individuals are more susceptible to attack by parasites and disease, such as the aquatic fungus <u>Saprolegnia</u>.

4 Reproductive potential may be altered. Eggs or fry may or may not be more susceptible to toxic substances than adults.

5 Host fish for mussel (unionidae) life cycle may not survive.

6 The result may be a slow and subtle alteration of the characteristics of a stream over an extended period of time.

C The specific physiological mechanisms involved are infinite in variety and but little known. Included are such processes as enzyme inhibition as in the case with some of the pesticides, and over stimulation of mucous membranes of the gills, leading to death by suffocation.

D There are a number of excellent diagnostic techniques for the examination of dying fish, these include pathogenic bacteria, parasites, some metals, and certain pesticides. None are routine but require specific handling and preservation techniques.

E A recently developed procedure for protecting aquatic life from deleterious substances is biomonitoring. This is the continuous monitoring or surveillance of an effluent for toxicity by means of a system for exposing living organisms (such as fish or invertebrates) to a continuously flowing stream in a dilution just below the known danger point. Should the toxicity of the substance increase the test organisms respond in some recognizable manner, thus giving warning that corrective measures need to be initiated.

VI MECHANISMS OF POLLUTION TOLERANCE AND SENSITIVITY

The fact that some organisms are more resistant to pollution than others needs no emphasis. The matter of "why?" and "how?" on the other hand, is quite another question. In some cases the answer is obvious, in others not. In general we can say that the adaptations of certain species enable them to resist certain types of natural conditions such as organic deposits or sand bars. When man artificially creates conditions such as sludge banks, or sand bars, organisms which can tolerate such conditions move in, survive, and often thrive. Other forms are eliminated.

A Organic pollution is essentially non-toxic. Its typical result as noted above is oxygen depletion, physical turbidity, and smothering blankets of sediment or sludge.

B Devices and mechanisms for living in oxygen-poor or oxygen-free water include the following

1 Obtaining oxygen from the air by means of periodic trips or access to the surface.

a The snorkel tube of the rat-tailed maggot.

b Periodic trips of the mosquito larvae and Corixidae or water boatmen. The mosquito takes air directly into its respiratory system, the water boatman into a trap or space beneath the wing covers, as well as into a layer of air held by fine hairs or "pile" all over its body.

c Behavior of the air breathing snails such as Physa which have an internal lung cavity.

d Insects which tap into air tubes in aquatic plants.

e Fishes which gulp air at surface or breathe surface water.

2 Special devices and behavior for respiration of water

a Hind intestine respiratory structures of dragonfly larvae permits respiration in silt-laden water.

b Movement of gill covers and similar structures in isopods and certain insect groups maintains a current of water over respiratory organs.

c Body movements of chironomid larvae create water current in tibe. Sludge worms and other annelids also create water movement by means of sinuous body movements.

3 Physiological and behavioral adaptations to endure low oxygen tensions.

a Forms possessing accessory respiratory pigments such as hemoglobin might be expected to be able to be able to extract the last vestige of dissolved oxygen from the water. Two groups famous for resisting low DO do have hemoglobin: the larvae of certain Chironomid midge flies, and small annelid worms such as sludge worms. (it should be noted however, that the hemoglobin in each case is simply dissolved in the blood plasma, rather than being concentrated in special corpuscles as is the case in the more efficient vertebrate system.)

b The mere possession of hemoglobin however, does not seem to assure tolerance of low DO (Walshe '47). Larvae of the midge Tanytarsus spp. have hemoglobin, but will not tolerate oxygen-poor waters. Hemoglobin-bearing Chironomus bathopilus is moderately tolerant, and Chironomus plumosus is highly tolerant, however.

c During periods of low DO, Chironomus plumosus apparently respires carbohydrates as usual, but excretes excess lactic acid instead of accumulating it.

d Various species of Daphnia (microcrustacea) have been shown to accumulate hemoglobin in oxygen-poor waters but not in oxygen-rich waters (Fox '47). No clear adaptive significance has yet been proven however.

C Advantageous Feeding Habits

All highly organic pollution tolerant organisms are scavengers, and hence find an abundance of food. Most are relatively defenseless and hence have normally high reproductive rate. The result in a polluted situation is thus usually an extreme abundance.

D The reverse problem is why are intolerant species intolerant?

1 A physiological requirement for higher oxygen levels is probably most basic.

2 Turbidity would hamper any organisms employing sight in any way.

3 Absence of light would suppress the growth of green algae, and hence also restrict the growth of algae feeders.

E Inert silts by themselves have many damaging effects such as abrasive or smothering action.

Biological mechanisms for enduring inert silt or sand pollution are not numerous, and consequently such locations are usually known as biological deserts. Since some life exists even in deserts however, a few forms may occasionally be found. In general they are typical sand or mud dwellers. Since the available food in such a substrate is at best of a very low order, inhabitants of these situations must either seek buried or trapped food particles or capture food from the passing waters.

1 If there is no BOD involved, and water and oxygen circulate down into the deposit, burrowing forms such as certain mayflies, annelid worms, ammocetes lamprey larvae, microcrustaceans, and others may burrow down to depths of two feet or more. Fish eggs normally deposited in gravel and newly hatched larvae are also dependent on circulating water. Such a population can be killed overnight by a layer of fine sediment or sludge which seals the surface to water circulation.

2 Water or plankton feeders include clams and mussels which can move about freely in a soft, shifting bottom, thus keeping on top of silt or sand as

it accumulates. If deposition is actively taking place, however, there will probably be so much turbidity in the water that plankton (food) organisms are unable to live. Under such circumstances certain clams and snails have the ability to close the shell so tightly by means of valves or an operculum that all contact is lost with the environment for extended periods, during which CO_2 tends to lower the rate of body metabolism. Organisms of this type have been reported to be dug up with sand and gravel and incorporated into concrete products while still alive. Emergence of a population of asiatic clams (Corbicula) for example, just as a big block of concrete is setting is said to be rough on contractors!

3 An interesting situation occasionally develops in estuaries where mud of moderate organic content is slowly deposited over oyster beds. The oysters are unable to move, but as they grow, their shells tend to bend upward above the accumulating silt, and may grow to several inches in length while growing very little in width. Crowding brings about a similar reaction in the effort to avoid being buried.

F Few generalizations can be offered relative to toxic pollution except that toxicity is relative, and all forms do not respond equally to a given toxicant.

1 Few mechanisms of toleration can be listed, beyond the natural resistance that certain forms may have for a given condition. For example, some marine species may survive a salt concentration that is toxic to freshwater species. Over a period of several generations, some species may develop a genetic resistance to some toxicant such as insecticides in the same way that DDT-resistant strains of houseflies have developed. Copper sulfate, and chlorine-resistant strains of algae such as Cosmarium for example, may develop in treated water supply reservoirs.

2 Some bottom in-fauna organisms such as annelid worms may retreat down into burrows until a slug of undesirable water passes.

3 Molluscs may close shells tightly for the same purpose. The metabolic rate is known to diminish with the increase of CO_2 inside the closed shell, thereby enabling them to remain tightly sealed for extended periods of time.

VII EFFECTS OF LIFE HISTORY STAGES

A In order to survive in a polluted area, each life history stage of an indigenous organism must be able to survive in turn.

B If some given life history stage cannot tolerate conditions, and the species is present:

1 Fortuitous changes may occur at the proper time(s) to permit survival of the more susceptible stages(s) or:

2 Recruitment from less polluted areas may occur.

C Some examples of reproductive stages or procedures which might affect pollution sensitivity.

1 Egg or egg-like stages are often enclosed in protective membranes, jelly masses, or cases. May remain dormant until favorable conditions develop.

2 Eggs may be deposited in locations where they are less exposed to polluted water as:

buried in the gravel,
on the surface film,
on rocks over the water moistened by spray,
on mud surface near water,
or in locations where maximum water circulation is encountered as at lip of waterfall.

3 Eggs may require minimal DO due to low metabolic rate.

4 Eggs deposited on or in bottom may be susceptible to smothering.

5 Newly hatched larvae often continue to live on stored yolk material for a time. On beginning to take natural food, they may be killed by toxic content thereof, such as organochlorines.

6 Some forms such as certain sludge worms commonly reproduce by (vegetative) fragmentation, hence avoiding egg and larva stages.

VIII NATURAL SELECTION AND ACCLIMATIZATION TO POLLUTION

A Known biological mechanisms for selective breeding of pollution resistant strains operate in nature among fishes as among other organisms.

1 Studies of population genetics indicate that after some finite number of generations of population stress (e.g., exposure to a given pollutant), permanent heritable resistance may be expected to develop.

2 If the environmental stress (or pollutant) is removed prior to the time that permanent resistance is developed in the population, reversion to the non-resistant condition may occur within a relatively few generations.

3 Habitats harboring populations under stress in this manner are often marked with the dead bodies of the unsuccessful individuals.

B Individual organisms on the other hand can over a period of time (less than one life cycle) develop a limited ability to tolerate different conditions, e.g., pollutants:

1 With reference to all categories of pollutants both relatively facultative and obligate species are encountered (e.g., euryhaline vs stenohaline, eurythermal vs stenothermal).

2 This temporary somatic acclimatization is not heritable.

C A given single-species collection or sample of living fishes may therefore represent one or more types of pollution resistance·

1 A sample of an original population which has been acclimated to a given stress in toto.

2 A sample of the surviving portion of an originalpopulation, which has been "selected" by the ability to endure the stress. The dead fish in a partial fish kill are that portion of the original population unable to endure the stress.

3 A sample of a sub-population of the original species in question which has in toto over a period of several generations developed a heritable stress resistance.

D Any given multi-species field collection will normally contain species illustrative of one or more of the conditions outlined above.

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THE EFFECTS OF ORGANISMS ON POLLUTION AND THE ENVIRONMENT

I INTRODUCTION

A Pollution is often studied as a factor affecting the biota, but it is equally important to recognize the environmental changes produced by the biota.

B According to Westlake, under many conditions "....the environment is almost as much a product of the community as the community is of the environment."

II SOME ENVIRONMENTAL EXAMPLES

A Diatom Blooms

"...Asterionella (an oil storage alga) produces an autotoxin (autoantibiosis) which will inhibit itself, may stimulate or inhibit other species that store oil, but always stimulate algae that store starch. For example, Asterionella may produce a bloom and inhibit itself, but stimulate a population of Synedra. These oil storage algae produce a substance that stimulates a starch-storing alga, Coelastrum, which may stimulate another starch-storing organism, Cosmarium, and they, in turn, stimulate an oil storing species of Dinobryon." Patrick.

B The altered structure of the plankton community due to the introduction of the alewife

C Fecal deposit feeders in the estuarine environment

D Particle feeders are successful in the pipe clogging community and are generally (Sebestyén)·

1 Sessile

2 Suspension feeders

3 Have motile larvae

4 Resistant stages

E Biogeochemical Cycles

In terms of biomass and energy flow, the mussel Modiolus (Figure 1) is a relatively minor component of the marsh community. However, they have been demonstrated to have a major effect on the recycling and retention of valuable phosphorus, thereby maintaining fertility and production of autotrophs (Odum).

F Sudd (dense aggregations of floating weeds)

Flowering aquatic plants are a serious problem in shallow, stagnant, or slow-flowing water in many tropical countries. They are expensive liabilities in newly impounded reservoirs in developing nations.

G Biological Pollution

Contamination of living native biotas by introduction of exotic life forms has been called biological pollution by Lachner et al. Some of these introductions are compared to contamination as severe as a dangerous chemical release. They also threaten to replace known wildlife resources with species of little or unknown value.

1 Tropical areas have especially been vulnerable. Florida is referred to as "a biological cesspool of introduced life".

2 Invertebrates

a Asian Clams have a pelagic veliger larvae, thus, a variety of hydro installations are vulnerable to subsequent pipe clogging by the adult clams.

b Melanian snails are intermediate hosts for various trematodes parasitic on man.

3 Vertebrates

a At least 25 exotic species of fish have been established in North America.

b Birds, including starlings and cattle egrets.

c Mammals, including nutria.

4 Aquatic plants

Over twenty common exotic species are growing wild in the United States. The problem of waterway clogging has been especially severe in parts of the Southeast.

III In polluted environments, there is a more noticeable change in balance, time response, and effects.

A Organic Pollution

The conditions here are classic and well described. There is a succession of biological communities (Figure 2) each of which modify the environment and water properties, thus, the effects are predominately biological.

1 Tubificidae (aquatic earthworms) in the first zone may reach as many as $1,7000,000/m^2$ and move up to 50 tons of mud per acre per day.

2 In the second zone chironomids (midge larvae) are found in thousands per square meter and may reduce the DO level by one and one-half ppm per stream mile.

3 The Isopod (sowbug) zone (the genus Asellus or Lirceus depending on locality) third in succession also may reach a density of thousands per square meter, a further oxygen demand due to respiration. (Figure 2)

4 The filamentous green alga Cladophora in both streams and lakes responds to organic enrichment by producing dense growths. (Figure 3)

5 Higher aquatic plants such as Potamogeton pectinatus may also be involved in these ecological changes in streams, particularly respiration vs. photosynthesis. There is correlation between weed bed growths, velocity and silt deposition. In some streams these massive growths on sloughing off foul water intakes and reduce DO levels as they decompose.

6 Sphaerotilus and/or slime growths below organic wastes, by metabolic demands while living and decomposition after death, impose a high BOD load on the stream and can severely deplete the dissolved oxygen. (Figure 3)

7 Blackflies (Diptera, family Simuliidae) often reach large populations below organic waste sources, filter feed on this material and in so doing, further degrade stream conditions with their fecal deposits.

B Inorganic and Toxic Pollution

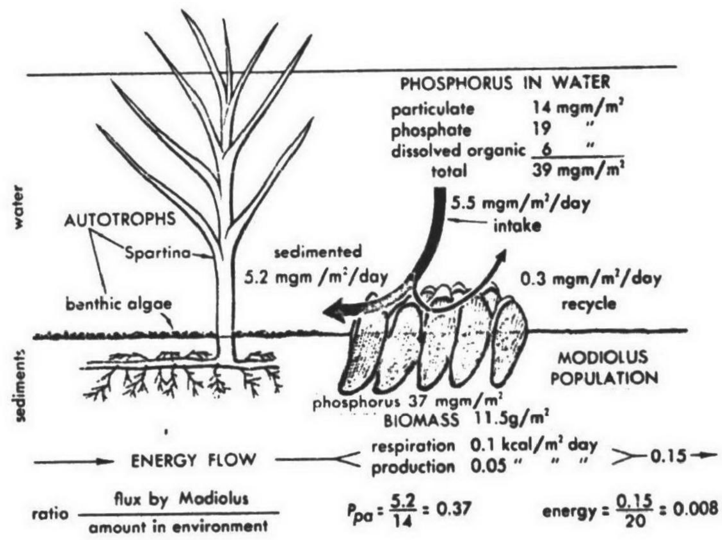
When progressive changes occur they are rarely produced by the biological community.

C Biological Magnification

Biological magnification is an additional chronic effect of toxic and other pollutants (such as heavy metals, pesticides, carcinogens, teratogens, radionuclides, bacteria, and viruses) which must be recognized and examined before clearance can be given for the disposal of a waste product into natural waters. To paraphrase Odum we could give nature an apparently innocuous amount of pollutant and have her give it back to us in a lethal or detrimental package.

1 Many animals, and especially bivalves, have the ability to remove from the environment and store in their tissues substances present at nontoxic levels in the surrounding water

a This process may continue in the clam or fish, for example, until the body burden of the toxicant reaches such levels that the animal's death would result if the pollutant were released into the bloodstream by physiological activity.



(Reproduced from Figure 4-4, from Ecology by Eugene P. Odum, copyright (c) 1963 by Holt, Rinehart and Winston, Inc. used by permission of Holt, Rinehart and Winston, Inc.)

Figure 1. The role of a shellfish (mussel) population in the cycling and retention of phosphorus in an estuarine ecosystem.

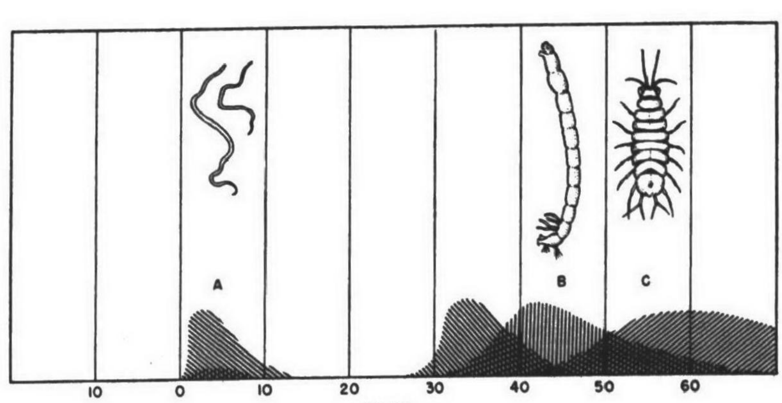


Figure 2. Linear alterations in populations of Tubificids (A) Chironomids (B), and Isopods (C) (from Bartsch). Zone C is often referred to as the Cladophora-Asellus Zone.

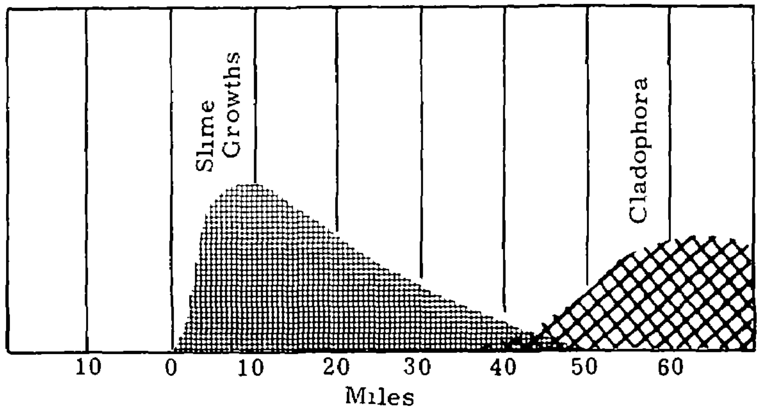


Figure 3. Linear alterations in populations of slime growths and Cladophora (modified from Bartsch)

b This may occur, as in the case of chlorinated hydrocarbon pesticides (such as DDT and endrin) stored in fat depots, when the animals food supply is restricted and the body fat is mobilized.

c The appearance of the toxicant in the bloodstream causes the death of the animal.

2 The biological magnification and storage of toxic residues of polluting substances and microorganisms may have another serious after effect.

a Herbivorous and carnivorous fish at lower trophic stages may gradually build up DDT residues of 15 to 20 mg/l without apparent ill effect.

b Carnivorous fish, mammals, and birds preying on these contaminated fish may be killed immediately or suffer irreparable damage because of the pesticide residue or infectious agent.

c Commercial shellfisheries are damaged by toxins produced by some dino-flagellate blooms in nearshore waters.

IV In summary, the biological causes of DO changes, associated changes in pH and CO_2, ammonia, nitrates, and sulphides, require study if the effects of organic pollution are to be calculated and predicted. Further biological effects on pollution include the relation to fouling organisms, stabilization of sediments in estuaries, recycling of nutrients, and problems of biological magnification.

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THE EFFECTS OF POLLUTION ON LAKES

I INTRODUCTION

The pollution of lakes inevitably results in a number of undesirable changes in water quality which are directly or indirectly related to changes in the aquatic community.

A Industrial Wastes may contain the following·

1 Sewage

2 Dissolved organics--synthetics, food processing wastes, etc.

3 Dissolved minerals--salts, metals (toxic and nontoxic), pigments, acids, etc.

4 Suspended solids--fibers, minerals, degradable and non-degradable organics

5 Petroleum products--oils, greases

6 Waste heat

B The Materials in Domestic Wastes which affect Water Quality are

1 Pathogenic fecal microorganisms

2 Dissolved nutrients. minerals, vitamins, and other dissolved organic substances

3 Suspended solids (sludge)--degradable and non-degradable organic materials

C Pollution and Eutrophication

The discharge of domestic wastes often renders the receiving water unsafe for contact water sports and water supplies. For example, some beaches on the eastern seaboard and in metropolitan regions of the Great Lakes are unfit for swimming because of high coliform counts. Other effects of domestic pollution include changes in the abundance and composition of populations of aquatic organisms.

1 As the nutrient level increases, so does the rate of primary production.

2 Shore-line algae and rooted aquatics become more abundant. For example, problems have been experienced with Cladophora and Dichotomosiphon along the shores of Lakes Ontario, Erie, and Michigan. These growths interfere with swimming, boating, and fishing, and cause odors when the organisms die and decay.

3 The standing crop of phytoplankton increases, resulting in higher counts and greater chlorophyll content. Increases in phytoplankton abundance may result in taste and odor problems in water supplies, filter clogging, high turbidity, changes in water color, and oxygen depletion in the hypolimnion.

4 Populations of fish and larger swimming invertebrates increase, based on the increase in basic food production.

5 Changes in dominant species

a Diatom communities give way to blue-greens. Toxic blue-greens may pose a problem.

b Zooplankton changes include replacement of Bosmina coregoni by B. longirostris.

c Trout and whitefish are replaced by perch, bass, and rough fish.

d Hypolimnion becomes anaerobic in summer, bottom sludge buildup results in loss of fish food organisms, accompanied by increase in density of sludgeworms (oligochaeta).

II HISTORICAL REVIEW

The cultural eutrophication of a number of lakes in Europe and America has been well documented.

A Zurichsee, Switzerland

1 1896 - sudden increase in Tabellaria fenestrata

2 1898 - sudden appearance of Oscillatoria rubescens which displaced Fragilaria capucina

3 1905 - Melosira islandica var. helvetica appeared

4 1907 - Stephanodiscus hantzschii appeared

5 1911 - Bosmina longirostris replaced B. coregoni

6 1920
1924 - O. rubescens occurred in great quantities

7 1920 - milky-water phenomenon, precipitation of $CaCO_3$ crystals (40μ) due to pH increase resulting from photosynthesis

8 Trout and whitefish replaced by perch, bass, and rough fish

B Hallwilersee, Switzerland

1 1897 - Oscillataria rubescens not observed up to this time

2 1898 - O. rubescens bloomed, decomposed, formed H_2S, killing off trout and whitefish

C Lake Windermere, England (core study)

1 Little change in diatoms from glacial period until recent times

2 Then Asterionella appeared, followed by Synedra

3 About 200 years ago, Asterionella again became abundant

4 Asterionella abundance ascribed to domestic wastes

D Finnish Lakes

Aphanizomenon, Coelosphaerium, Anabaena, Microcystis, are the most common indication of eutrophy.

TABLE 1 CHANGES IN PHYSIO-CHEMICAL PARAMETERS

Zurichsee, Switzerland

Parameter	Date	Value
Chlorides	1888	1.3 mg/l
	1916	4.9 mg/l
Dissolved organics	1888	9.0 mg/l
	1914	20.0 mg/l

		Max.	Min.
Secchi Disk	before 1910	16.8M	3.1M
	1905 - 1910	10.0M	2.1M
	1914 - 1928	10.0M	1.4M
Dissolved oxygen, at 100 M, mid-summer	1910 - 1930	Minimum	100% saturation
	1930 - 1942	"	.9% saturation

E Linsley Pond, Connecticut

1 Species making modern appearance include Asterionella formosa, Cyclotella glomerata, Melosira italica, Fragilaria crotonensis, Synedra ulna

2 Asterionella formosa and Melosira italica were considered by Patrick to indicate high dissolved organics

3 Bosmina coregoni replaced by B. longirostris

F Lake Monona, Wisconsin

1 Began receiving treated sewage in 1920, developed blue-green algal blooms.

G Lake Washington, Washington

1 1940 - Bosmina longirostris appeared

2 1955 - Oscillatoria rubescens seen for the first time, and constituted 96% of phytoplankton, July 1

H Lake Erie

1 Phytoplankton counts at Cleveland have increased steadily from less than 500 cells/ml in the 1920's to over 1500 cells/ml in the 1960's

2 Abundance of burrowing mayflies (Hexagenia spp.) in Western Lake Erie decreased from $139/m^2$ in 1930, to less than $1/m^2$ in 1961.

I Lake Michigan

1 Milky water observed in south end, and in limnetic region in mid-1950's and again in 1967.

2 During the period 1965-1967 the Chicago water treatment plant has found it necessary to increase the carbon dosage from 23 lbs/mil gal to 43 lbs/mil gal, and the chlorine dosage from 20 lbs/mil gal to 25 lbs/mil gal.

3 Phytoplankton counts in the south end now exceed 10,000/ml during the spring bloom.

III FACTORS AFFECTING THE RESPONSE OF LAKES TO POLLUTION INCLUDE.

A Depth-surface area ratio· A large hypolimnion will act as a reservoir to keep nutrients from recirculating in the trophogenic zone during the summer stratification period. Rawson found an inverse relationship between the standing crop of plankton, benthos, and fish, and the mean depth.

B Climate· Low annual water temperatures may restrict the response of the phytoplankton to enrichment.

C Natural color or turbidity: Dystrophic (brown-water) lakes may not develop phytoplankton blooms because of the low transparency of the water.

IV TROPHIC LEVEL

Except in cases where massive algal blooms occur, the trophic status of lakes is often difficult to determine. Core studies are used to determine trends in diatom populations which might indicate changes in nutrient levels over an extended period of time.

V CONTROL OF POLLUTION

The success of efforts to arrest the eutrophication process, and where desirable, reduce the trophic level of a lake, will depend on a thorough knowledge of the nutrient budget.

A Significant quantities of nutrients may enter a lake from one or more of the following sources·

1 Rainfall

2 Ground water

TABLE 2 PARAMETERS COMMONLY USED TO DESCRIBE CONDITIONS

	Oligotrophic Condition
1 Transparency	$\geq$ 10 meters
2 Phosphorus	$\leq$ 1µg/l
3 NO_3 - Nitrogen	$\leq$ 200 µg/l
4 Minimum annual hypolimnetic oxygen concentration	near 100% saturation
5 Chlorophyll	$\leq$ 1 mg/m^3
6 Ash-free weight of seston	$\leq$ 0.1 mg/l
7 Phytoplankton count	$\leq$ 500/ml
8 Phytoplankton quotients	
a $\frac{\text{number of species of Chlorococcales}}{\text{number of species of Desmids}}$	<1
b $\frac{\text{Myxophycease+Chlorococcales+Centrales+Euglenaceae}}{\text{Desmidaceae}}$	<1
c $\frac{\text{Centrales}}{\text{Pennales}}$	0 - 0.2
9 Phytoplankton species present (see outline on plankton in oligotrophic lakes).	

3 Watershed runoff

4 Shoreline domestic and industrial outfalls

5 Pleasure craft and commercial vessels

6 Waterfowl

7 Leaves, pollen, and other organic debris from riparian vegetation

B The supply of nutrients from "natural" sources in some cases may be greater than that from cultural sources, and be sufficient to independently cause a rapid rate of eutrophication regardless of the level of efficiency of treatment of domestic and industrial wastes.

C Many methods have been employed to treat the symptoms, reduce the eutrophication rate, or completely arrest and even reverse the eutrophication process.

1 Use of copper sulfate, sodium arsenite, and organic algicides· It is not economically feasible to use algicides in large lakes.

2 Addition of carbon black to reduce transparency. This is likewise frequently impractical.

3 Harvesting algae by foam fractionation or chemical precipitation.

4 Reducing nutrient supply by (a) removal of N and P from effluents, (b) diversion of effluents, and (c) dilution with nutrient-poor water.

D Examples of lakes where control has been attempted by reducing the nutrient supply, are·

1 Lake Washington, Seattle

The natural water supply for this lake is nutrient poor (Ca = 8 mg/l, $P \leq 5$ µg/l, TDS = 76 mg/l). Since the turnover time of the water in this lake is only three years, it was expected that diversion of sewage would result in a rapid improvement of water quality. Diversion began in 1963, and improvements were noticeable by 1965 - including an increase in transparency, and a reduction in seston, chlorophyll, and epilimnetic phosphorus.

TABLE 3

PHOSPHORUS REDUCTION IN LAKE WASHINGTON

Year	Maximum phosphorus in upper 10 meters (µg/l)
1963	70
1964	66
1965	63

2 Green Lake, Washington

The lake has a long history of heavy blooms of blue-green algae. Beginning in 1959, low-nutrient city water was added to the lake, reducing the concentration of phosphorus by 70% in the inflowing water. By 1966, the lake had been flushed three times. Evidence of improvement in water quality was noted in 1965, when Aphanizomenon was replaced by Gleotrichia.

3 Lake Tahoe

This lake is still decidedly oligotrophic. To maintain its high level of purity, tertiary treatment facilities were installed in the major sewage treatment plant, and construction is now underway to transport all domestic wastes out of the lake basin.

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WATER TEMPERATURE AND WATER QUALITY

I INTRODUCTION

A Temperature is the basic variable in water "climates".

1 Temperature, or the amount of thermal energy present, is originally of solar or cosmic origin. Biological processes acting over geologic time temporarily capture and store much energy in organic substance. The "fossil fuels" (oil, gas, and coal) which we burn today release solar energy captured in the geologic past, which but for man might not have been released until sometime in the geologic future. Most of the solar energy being captured today, is released probably today, with the possible exception of some unrecognized fraction which is proceeding into long term storage.

The release of atomic energy is of inorganic or cosmic origin, and the magnitude and significance of the additional thermal discharge to the environment has yet to be accessed.

One last observation is important before turning to the details of water temperature and water quality. While we are most critically occupied with the immediate or local impact of a concentration of thermal energy released at a given point in the environment (the excess or "waste" which man is unable to capture and entrain in his electric transmission lines), it should be remembered that of every ton of coal or pound of uranium burned as fuel, nearly 100% of the energy contained eventually finds its way into the general global environment. A small remaining fraction is rebound as chemical energy in some "product."

2 Direct solar radiation is the overriding contributor of thermal energy to all lands and waters.

a Total energy from insolation onto "spacecraft earth" is counterbalanced by the radiation of terrestrial energy into space. If the two do not exactly balance on an annual basis, the overall temperature (climate) of the earth will rise or fall.

b The annual climate or heat budget of a given body of water is determined by its geographic location (latitude elevation, etc.) interacting with local meteorological conditions, and other factors.

c There is, therefore, a natural or normal temperature regimen for any given body of water to which it will tend to return if disturbed by man.

d There is, also, a normal or characteristic community of aquatic organisms that will tend to persist. When the heat budget or climate of a body of water is changed, the fauna and flora change.

3 There is great diversity of opinion, even among knowledgeable people, as to the effects of thermal changes in waters. Some of the reasons for this follow:

a Only a continuously maintained temperature of 100° C could keep a surface water mass sterile. The question is therefore not life or no-life; but rather: what kind of life is the objective?

b Aquatic life has received more attention than other water uses because the aquatic organisms cannot escape the water conditions.

c There are certain circumstances in which a modest rise in temperature might be considered to be beneficial as for example: keeping an area of a river or a harbor free of ice for navigation, or winter fishing. There has, also, been investigation of the use of warmed waters for certain aspects of aquaculture.

d It is, therefore, important in discussing the virtues and vices of thermal changes to clearly define or specify the objective or type of aquatic community in mind.

4 It is clear that the need for more and more power will continue into the foreseeable future (Table 1).

B Human activities which may modify receiving water temperatures include the following·

1 Logging and other land stripping activities which increase the rate of surface run-off and hence raise or lower temperatures of influent waters, depending on the season.

2 Removal of stream bank shade

3 Erosion which fills in stream bed and causes water to be spread in broad shallow layer, exposed to sun and air.

4 Release of cold waters from hypolimnion of deep reservoirs.

5 Withholding or augmenting flow by dam manipulation.

6 Release of relatively large volumes of high temperature wastewaters from power production and/or industrial processes.

II THERMAL ELECTRIC POWER PRODUCTION AS A STREAM WARMING ELEMENT

A Production of electric power by stream plants involves the wastage of considerable quantities of energy in cooling waters. Approximately 5000 BTU of heat are wasted for each kilowatt of electricity generated. This represents an efficiency of roughly 40%.

B It is estimated that approximately 80% of all energy required in the future will come from steam generating plants.

C Weirs and jetties help greatly in the dispersal of warmed waters, but must be carefully designed to each situation.

D As water temperature rises, its value as a coolant diminishes.

E Heat dissipation from a body of water which has been heated above its equilibrium temperature with the meteorological conditions follows Newton's Law of Cooling which states that the rate of cooling is proportional to the difference between the temperature of the body of water, and the equilibrium temperature for the given meteorological conditions. For example, an analysis of the Ohio River as at Cincinnati has shown that it would require over 200 miles to dissipate 99 + % of heat added (Figure 1).

III EFFECTS OF HEAT ON ORGANIC WASTE DISPOSAL

A Higher temperatures accelerate the rate of bacterial growth, the optimum temperature being in the range of 30° C (86° F).

1 As water temperatures approach this value, the rate of BOD thus approaches a maximum.

TABLE 1

MAXIMUM TEMPERATURES PROBABLY COMPATIBLE WITH THE WELL-BEING OF VARIOUS SPECIES OF FISH AND THEIR ASSOCIATED BIOTA IN °C

Temperature	Taxa
34 C	Growth of catfish, gar, white or yellow bass, spotted bass, buffalo, carpsucker, threadfin shad, gizzard shad
32 C	Growth of largemouth bass, drum, bluegill, crappie
29 C	Growth of pike, perch, walleye, smallmouth bass, sauger, California killifish, topsmelt
27 C	Spawning and egg development of catfish, buffalo, threadfin shad, gizzard shad, California grunion, opaleye, northern swellfish
24 C	Spawning and egg development of large-mouthed bass, white and yellow bass, spotted bass, sea lamprey, alewife, striped bass
19 C	Growth or migration routes of salmonoids and for egg development of perch, small-mouth bass, winter flounder, herring
12 C	Spawning and egg development of salmon and trout (other than lake trout)
9 C	Spawning and egg development of lake trout, walleye, northern pike, sauger, and Atlantic salmon

2 If the waste assimilative capacity of a stream is being utilized based on a given stream temperature, and the temperature subsequently raised, the DO may drop so low as to produce fish kills and other nuisance conditions.

B Higher water temperatures may, also, lead to a higher concentration of bacteria pathogenic to man.

IV EFFECTS OF HEAT ON FISH AND OTHER AQUATIC LIFE

A Vulnerability of fish and other aquatic life to high temperatures represents a major restriction on the discharge of cooling water.

1 Involves duration of exposure as well as absolute thermal level.

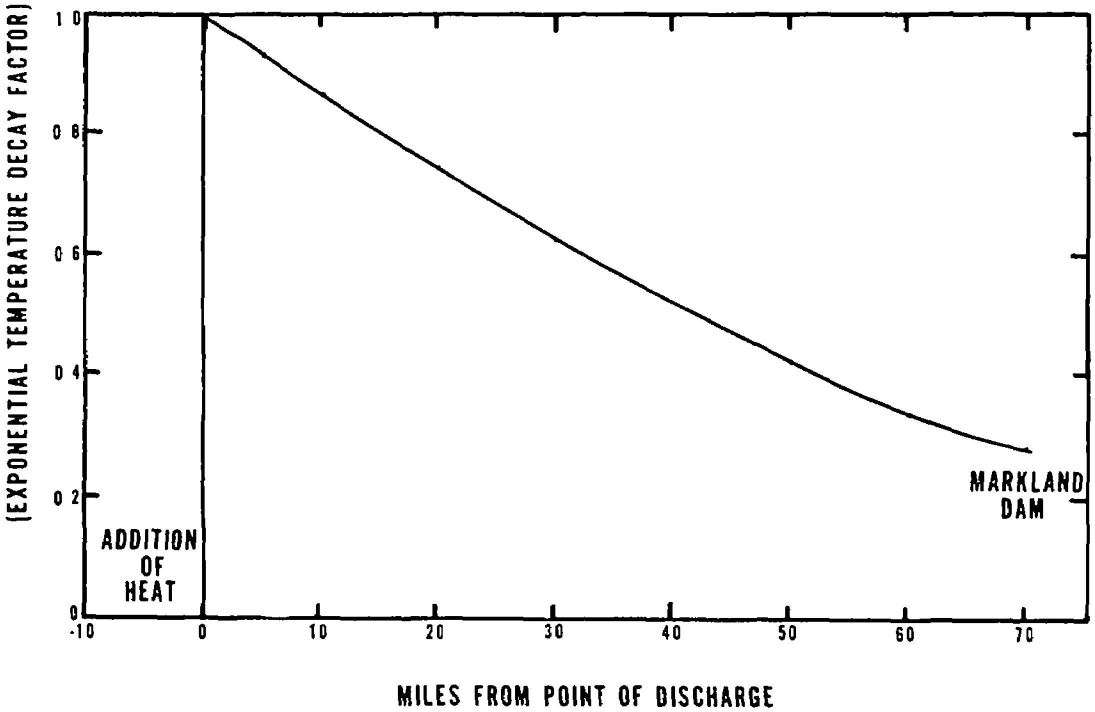
2 Sensitivity to toxic substances is increased.

3 Lower temperatures are required in winter than in summer.

B Factors contributing to the sensitivity of fish to heat

1 Oxygen solubility diminishes as temperature rises (Figure 2).

2 Oxygen requirements of aquatic life increase as temperature rises (Figure 3).



TEMPERATURE DROP THROUGH MARKLAND POOL
OHIO RIVER

Figure 1

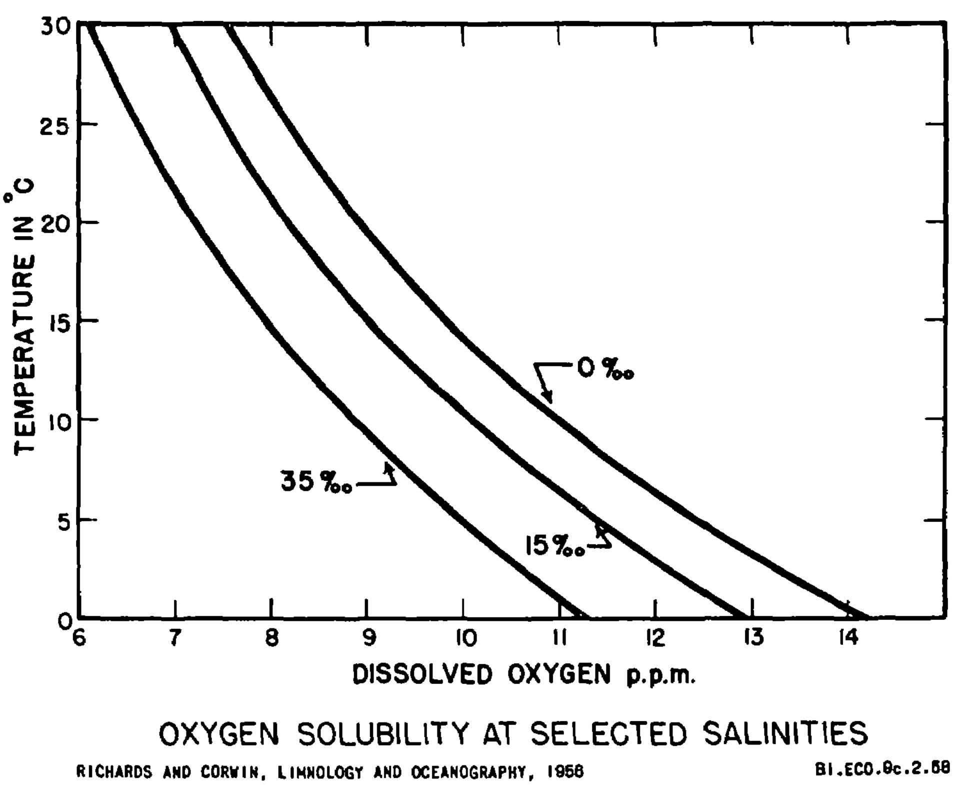
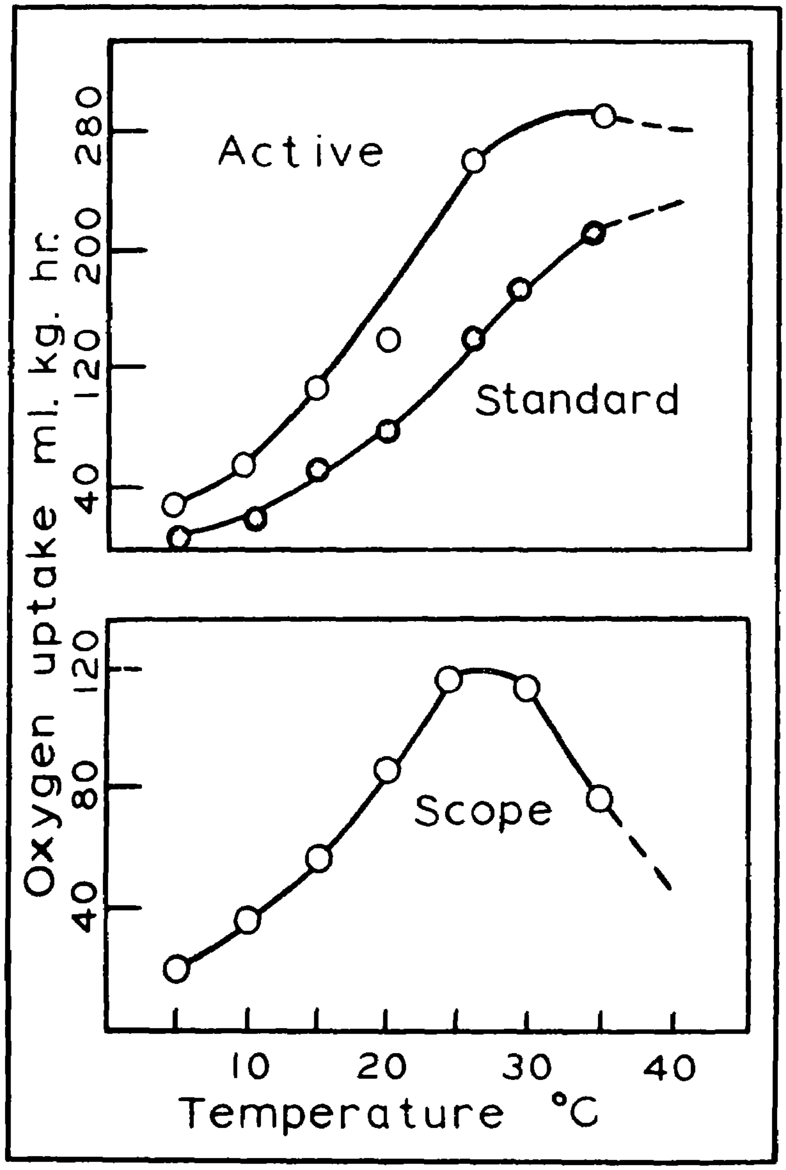


Figure 2

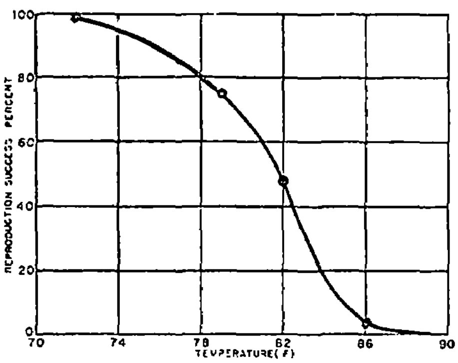


THE RELATION OF TEMPERATURE TO ACTIVE AND STANDARD METABOLISM IN YOUNG GOLDFISH OF AN AVERAGE WEIGHT OF 2 GM. From Fry and Hart (1948)

Figure 3

3 The sensitivity of aquatic organisms to temperature levels and changes varies with age, size, and size.

a A constant elevated temperature reduces the potential of fish to reproduce (Figure 4).



EFFECTS OF CONSTANT TEMPERATURE ON REPRODUCTION OF A MINNOW (*Pimephales promelas*)

Figure 4

b Different species have different preferred temperature ranges.

c Seasonal cooler temperatures are often essential to egg production and hatching, while warmer summer temperatures will promote faster growth after hatching, up to some limit of tolerance characteristic of the species.

4 Lethal high temperatures as determined in laboratory tests vary widely for different species, e.g. goldfish, 107.6° F, pink salmon· 75° F.

a Lethal temperatures differ at different times of the year, as well as for the different life history stages (Figure 5).

b This is analogous to a temperature of 50° F for man· in winter it feels "warm," in summer it is "cool."

5 Sudden changes in water temperature can be fatal to certain organisms, both fish and fish food organisms.

6 Acclimatization to higher temperatures is faster than to lower. Fish acclimated to warm water are rapidly killed when they swim into cold water. This implies that the sudden shutdown of a thermal discharge may be more detrimental than a continuous normal discharge.

7 Reduction in DO, increase in CO_2, or the presence of toxic materials reduces maximum tolerable temperatures.

8 Species can be eliminated at less than lethal temperatures by predators, parasites, or diseases which are less temperature-sensitive.

9 Some fish do not seem to be able to avoid killing hot waters, while others do.

10 Preferred temperature ranges in laboratory tests generally are somewhat higher than in field observations (Figure 6). This may be influenced by the demands of the natural environment for greater activity and hence a need for more oxygen (Figure 2, Table 1).

11 Temperature can act as a directive force in fish migration.

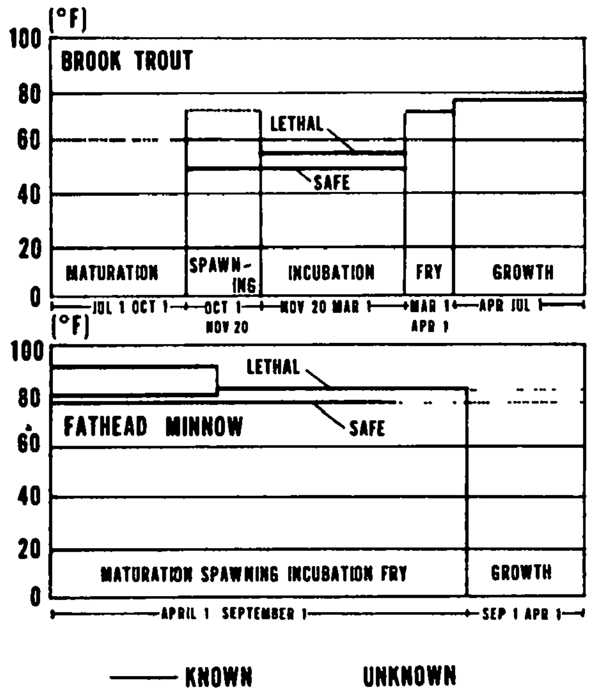
12 The exact physiological mechanisms of heat kill are not fully understood.

Fats rather than proteins seem to be the most critical substance.

a Some fish will die at temperatures of 65° F, lower than that at which proteins usually coagulate.

b Tropical (or heat-adapted) plants and animals often have fats with a higher melting point than arctic (or cold-adapted) organisms.

c Animals (such as goldfish) fed high melting point fats (e.g. beef) develop higher melting point body fats than those fed on low-melting fats (such as fish oil). They are in turn able to tolerate higher temperatures.



THERMAL TOLERANCE OF CRITICAL LIFE HISTORY STAGES

Figure 5

d Lethal temperatures seem to destroy fat-calcium relationships.

C Effects of Temperature on Fish Food Organisms

1 Species composition and abundance are affected in ways similar to the fishes as outlined above.

2 Warm waters encourage blue-green algae. Some can tolerate as high as 185° F for limited periods (Figure 7).

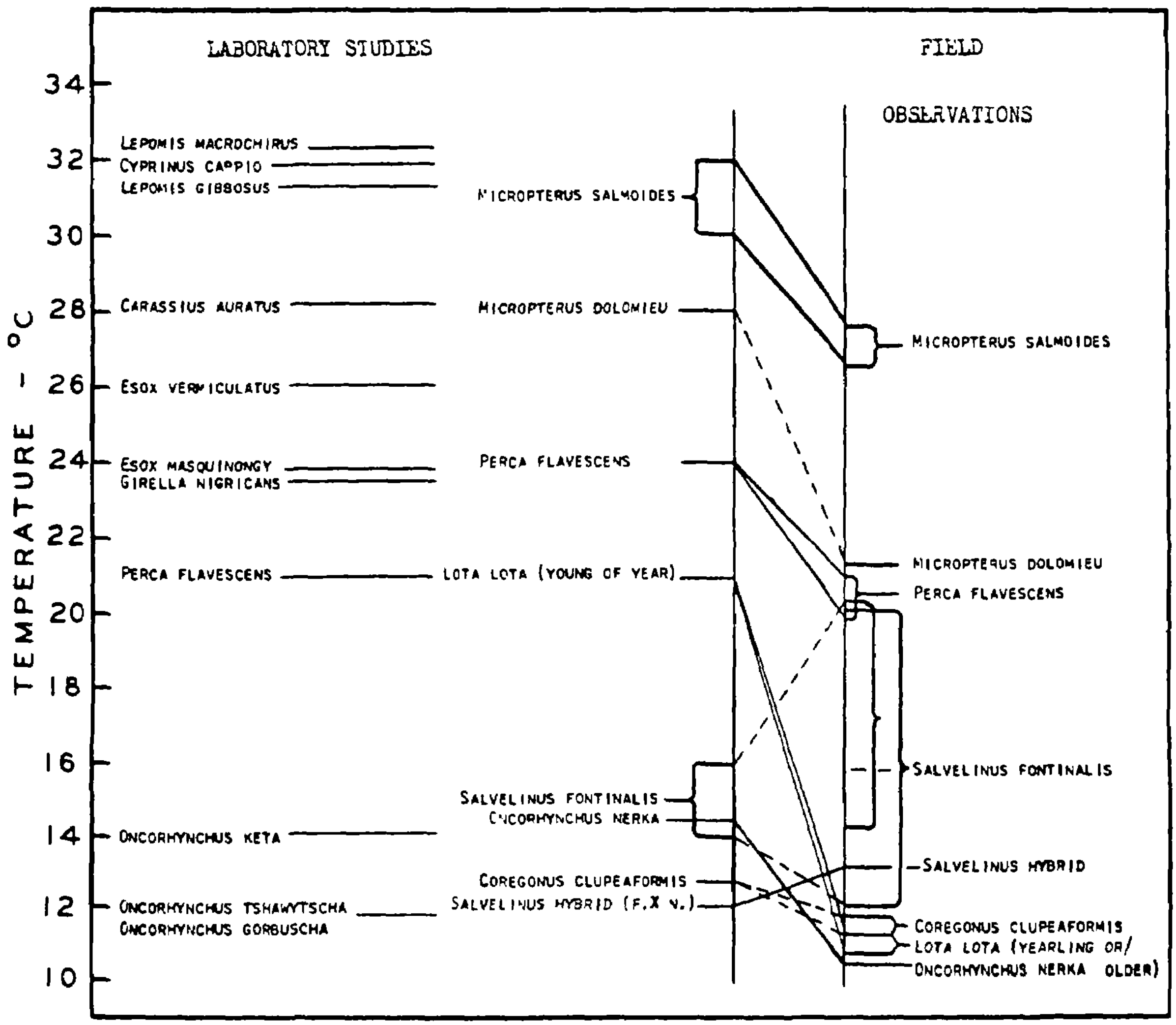
3 A temperature increase of 8° C stimulated photosynthesis in phytoplankton in one series of observations when ambient water temperatures were 16° C or cooler, but inhibited photosynthesis when natural waters were 20° C or warmer. The existence of a diurnal response to thermal stimulation was noted at 9:00 A.M.

4 Warmed waters speed up life cycles and encourage year round emergence of aquatic insects, often creating local nuisance conditions.

V MARINE, ESTUARINE, AND ANADROMOUS SPECIES

A The general principles of biotic responses to thermal conditions outlined above apply as well to salt water forms as to fresh.

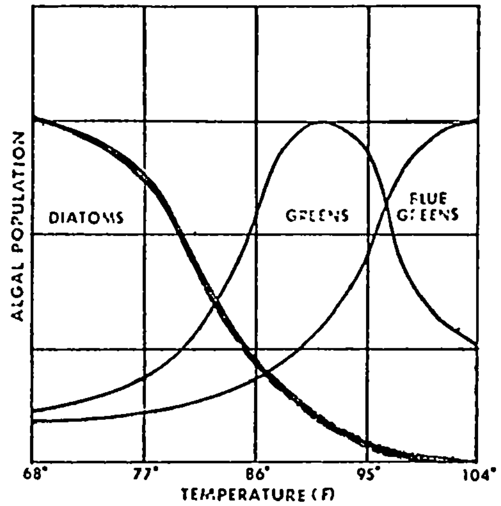
B Salmon do not feed during the spawning migration, hence higher temperatures may so increase their metabolic demands as to deplete their food reserves before spawning can occur. A thermal block at the mouth of a river (e.g. 21.1° C at the mouth of the Okawogan River in Washington) can prevent an entire spawning run.



Left. A comparison of various final preferenda as found by laboratory studies.

Right. A comparison of field observations with laboratory results for a number of species.

Figure 6



EFFECT OF TEMPERATURE ON TYPES OF PHYTOPLANKTON

Figure 7

1 The American Oyster Crassostrea Virginica may spawn, depending on its condition, at temperatures from 15 to 34° C, spawning being triggered by a rise in temperature.

2 The shore crab Carcides Maenas thrives, but does not breed at temperatures between 14 and 28° C. Breeding can only take place outside the heated area.

C Fish in the estuarine environment are more susceptible to temperature changes than those in fresh water. However, wider ranges of tolerance between species exist.

D Most shellfish (in the broad sense: mollusca and crustaceans) are relatively or highly stenothermal (unadaptable to rapid temperature changes). Some are stenothermal for one stage (e.g. spawning), and eury-thermal for others (e.g. growing).

Preliminary observation, also, indicates that heat stress may stimulate oysters to accumulate copper (as other stressful factors are known to do) without being a direct killing agent.

E The distribution of many benthic invertebrate organisms is temperature dependent (See Table 2).

ENVIRONMENTAL TEMPERATURE RANGES OF SOME MARINE INVERTEBRATES

Table 2

Taxa	Temperature range in ° C
American Oyster	4 - 34
European Oyster	0 - 20
Opossum Shrimp	0 - 31

F Observations in Miami, Florida indicate that the following groups of larger plants may show temporary or permanent changes following thermal discharge:

1 The sea grass Thalassia, an important habitat for invertebrates and stabilizes of the substrate.

2 Certain macro-algae

(Lurencia, Fucus, Laminaria, Macrocystis, Halimeda, and Acetabularia)

3 The phytoplankton (see Figure 7)

4 The epiphytic micro-algae

5 The benthic micro-algae

G The upper limits of thermal tolerance for two species of copepods from Chesapeake Bay were found to be near the normal temperature of the habitat during the summer. The addition of chlorine to the cooling water killed all copepods passing through the system at temperatures below the upper limits of thermal tolcrance.

Dry weight of total estuarine epifauna production averaged 2.8 times greater in the discharge canal than in the intake area over a 5 year period in another study.

VI SUMMARY

The various environmental factors cannot be considered as isolated entities, organisms respond to the entire environment. Temperature criteria thus must be based on the requirements of the entire aquatic population, and on the life history requirements for different seasons of the year.

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EFFECTS AND CONTROL OF OIL POLLUTION

I INTRODUCTION

The increased size of oil tankers, density of waterborne traffic, and offshore petroleum production operations point up the need for prevention and control of oil spillage. For example, the Torrey Canyon wreck dumped upwards of 30 million gallons of oil on the coast of England in 1967, the Ocean Eagle wreck spread over three million gallons in San Juan Harbor alone. Other major spills also occurred in the York River, Virginia, and on the Cape Cod National seashore in 1967, and the well-known Santa Barbara, California, oil well incident contaminated over 400 miles of coastline.

A Specific Areas of Interest

1 On-scene control of gross leakage

2 Destruction or recovery of open sea or open water oil slicks

3 Disposal of recovered mixtures

4 Protection of the shore face and estuaries

5 Cleaning of shore face and estuaries

6 Effect of oil pollution and treatment agents on marine and freshwater flora and fauna

7 Waterfowl recovery methods

B Floating Sorbers and Sinkants

1 Examples of collecting agents

a Floating sorbers such as straw, or mineral perlites

b Plastic or other polymeric materials such as polyurethane foam

c Gelling agents

2 Floating Sorbers

a Inexpensive

b Can be disposed of by burning or burial

c Oil recovery very difficult

3 Plastics (burning not recommended)

a Expensive

b Excellent solution to problem since no residues are left on the bottom

c Large quantities of oil can be reclaimed for subsequent use

4 Gelling Agents

a In developmental stage

b May prove helpful in collecting spilled oil

5 Sinkants

a Sink oil, such as by certain chalks or carbonized sand

b Demersal fish species may be affected adversely

c Resurfacing of oil mass generally is probable, although it is delayed and slow

d Method should be applied only beyond continental shelf and in areas not involving commercial fisheries

6 The spreading of sorbers, polymeric materials, gelling agents, etc., is aided when spread by specialized equipment.

7 Confined slicks are easier to handle

C Chemical Treatment

1 A large number of dispersants and emulsifiers exist, although little quantitative or comparative information on toxicities and effectiveness to aquatic life is available.

2 The aromatic oil solvents used in the compounding of many of these agents have been found to be the principal toxic agent.

3 Based on review of the Torrey Canyon incident, opinion is divided on the general use of oil emulsifiers when water pollution control is of prime consideration. *

D Biological Degradation of Crude Oil and Oil Fractions in the Ocean and Fresh Water

1 Biological degradation is controlled by the following environmental conditions.

a Nutrients

b Temperature

c O_2 availability

d Degree of dispersion of oil

e Species of bacteria

f Other species of invertebrates

2 Degradation rates are very slow.

3 Seeding of oil slicks with micro-organisms which feed specifically on the oil requires further research.

E Booming

1 The ability to confine a spill is principally a function of time, availability and efficiency of equipment, and prevailing environmental conditions.

2 If oil release occurs over several days, the ability to confine the oil to the immediate area would depend largely on the prevailing water conditions

a Booming in waters with a sea state greater than three is impractical with most of the presently existing boom designs.

b Even with a sea state of three or less, this is suspect if wind conditions are adverse.

c Currents in excess of 1.5 to 2 knots make booming difficult without extensive skirts and anchoring systems

3 There are two principal types of mechanical barriers applicable to oil spills--floating booms and underwater bubble barriers.

a Both presently are suitable only for calm water and are subject to failure

b The floating boom is generally superior to the bubble barrier on an emergency basis as it is more portable

c The main advantage of a bubble barrier is the unrestricted entry and exit of ships

*Review Dept. of Interior policy on use of chemicals to treat oil (in lack of national contingency plan) available as handout, or from Dept. of Interior.

d The main disadvantage is the complete loss of containment in the event of air supply failure

F Burning

1 If all attempts to salvage a vessel or cargo have failed and a ship has been abandoned, an attempt should be made to set the oil afire while still contained in the vessel.

2 Burning on the surface generally is not effective due to the rapid transfer of heat to the water, selective burning of lighter-fractions, and lack of O_2 supply except at the edge of a slick.

3 Caution should be exercised in the vicinity of ships, docks, etc.

G Skimming

1 Mechanical devices for collecting oil from the surface of water, such as rotating cylinders and suction pumps, generally are available but have relatively small capacities

2 The use of these devices is restricted to relatively calm waters if reasonable efficiency is to be achieved

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BIOTIC EFFECTS OF SOLIDS

I Sedimentation of rivers, lakes, estuaries and adjacent coastal water should be considered as a special case of pollution resulting from deforestation, overgrazing and faulty agricultural practices, road construction, and other land management abuses.

A Good farming practices can do a great deal to prevent silt from reaching streams and lakes.

B Road building and housing development projects, placer mining, strip mining, coal and gravel washing, and unprotected road cuts are important sources of turbidity that can be reduced with planning, good housekeeping, and regulation.

II Setteable solids include both inorganic and organic materials which may settle out rapidly, forming bottom deposits of both inorganic and organic solids.

A They may adversely affect fisheries by covering the bottom of the stream or lake with a blanket of material that destroys the bottom fauna or the spawning grounds of fish (Figure 1 from Ingram, et al).

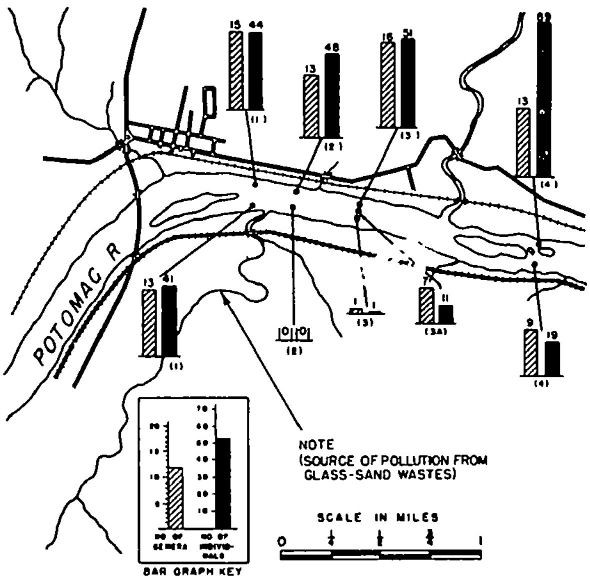

Figure 1 Vertical bar graphs, superimposed over a map, used to show total genera and individuals of bottom animals per unit area

B The organic fraction includes such setteable materials as greases, oils, tars, animal and vegetable fats, feed lot wastes, paper mill fibers, synthetic plastic fibers, sawdust, hair, greases from tanneries, and various settleable materials from city sewers. Deposits containing organic materials may deplete bottom oxygen supplies and produce hydrogen sulfide, carbon dioxide, methane, or other noxious gases.

C The inorganic components include sand, silt, and clay originating from such sources as erosion, placer mining, mine tailing wastes, strip mining, gravel washing, dusts from coal washeries, loose soils from freshly plowed farm lands, highway, and building projects.

D Some settleable solids may cause damage by mechanical action.

E The biota of streams is limited by the type of substrate.

1 A depositing substrate generally contains fewer types and may be dominated by burrowing forms.

2 An eroding substrate has a characteristic fauna of sessile attached and foraging members, such as bryozoans, stoneflies, nonburrowing mayflies, and net-spinning caddis flies.

3 The addition of solids over an originally eroding riffle substrate brings about pronounced changes in the biological community from diatoms to fish. The following are common macroinvertebrates of this new "trickling filter" community in contrast to E 2 above.

a Oligochaetes

b Alderfly larvae (Sialis)

c Midge larvae (Chironomids)

III Turbidity, color, and transparency are closely interrelated phenomena in water. They must be observed simultaneously because transparency is a function of turbidity, water color, and spectral quality of transmitted light.

A Turbidity is an expression of the optical property of a sample of water which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.

B Turbidity is caused by the presence of suspended matter such as clay, silt, finely divided organic matter, bacteria, plankton, and other microscopic organisms.

1 Algae, turbidity from silts and clays, and color of the water all affect one environmental factor of major importance in the productivity of aquatic wildlife habitat--light penetration of the water.

a Excessive turbidity reduces light penetration in the water and, therefore, reduces photosynthesis by phytoplankton organisms, attached algae, and submersed vegetation.

b The results of many of man's activities, including agriculture, industry, navigation, channelization, dredging, land modification, and eutrophication from sewage or fertilizers, often reduce light transmission to the degree that aquatic angiosperms of value to wildlife cannot grow.

c Mixed effluents from various industrial plants and domestic sewage increase the turbidity of receiving water. It is difficult to distinguish between the effect of the attenuation of light due to suspended particles and the direct effect of the particles in suspension on the growth physiology of aquatic organisms.

2 In many coastal waters, the principal cause of turbidity is the discharge of silt carried out by the principal rivers. Secchi disc readings show that the transparency of water at the mouths of large rivers during flood stage may be reduced to a few centimeters. At normal river stages, the disc may be visible at several meters below the surface.

3 Dredging of bays and tidal rivers for improvement of navigation occasionally presents serious problems. Benthic communities in the area near dredging operations may be destroyed or damaged by spoil deposition, increase in water turbidity, release of toxic substances accumulated in the mud of the polluted areas, and by changing the pattern in the dredged area.

IV PHYSICAL DAMAGES FROM SILTATION

A Silt and sediment are particularly damaging to gravel and rubble-type bottoms. The sediment fills the interstices between gravel and stones, thereby eliminating the spawning grounds of fish and the habitat of many aquatic insects and other invertebrate animals such as mollusks, crayfish, fresh water shrimp, etc.

B Accumulation of silt deposits is destructive to marine plants, not only by the associated turbidity, but by the creation of a soft, semi-liquid substratum inadequate for anchoring the roots. Back Bay, Virginia and Currituck Sound, North Carolina serve as examples of the destructive nature of silt deposition. Approximately 40 square miles of bottom are covered with soft, semi-liquid silts up to 5 inches deep; these areas, constituting one-fifth of the total area, produce only 1 percent of the total aquatic plant production.

V SILT POLLUTION INCLUDES NOT ONLY PURELY PHYSICAL EFFECTS, BUT ALSO MAY INCLUDE COMPLEX MATERIAL.

A Pollution in the estuary may be derived from contamination hundreds of miles upstream in the river basin or it may be of purely local origin. Silt plays a major role in the transport of toxicants, especially pesticides, down to the estuary.

1 Agricultural chemicals are adsorbed on silt particles. Under poor farming practices, as much as 11 tons of silt per acre per year may be washed by surface water into a drainage basin.

2 Surface mining and deforestation further accelerate the process of erosion and permit the transport of terrestrial chemical deposits to the marine environment.

B Oil that settles to the bottom of aquatic habitats can blanket large areas and destroy the plants and animals of value of waterfowl.

1 Reportedly, some oil sludges on the bottoms of aquatic habitats tend to concentrate pesticides, thus creating a double hazard to waterfowl that would pick up these contaminants in their normal feeding process.

2 Observations on storage of carcinogenic compounds found in oil-polluted water and on affected sediments are biologically significant, since they may be concentrated by commercially harvested bivalves.

C Much of the tonnage of aerially applied pesticides fails to reach the designated spray areas and the presence of 5 μg/l of DDT in presumably untreated Alaskan rivers indicates the magnitude of this facet of the pollution problem.

1 The continuous presence of 5 μg/l of DDT in the marine environment would decrease the growth of oyster populations by nearly 50 percent.

2 Atmospheric drift is also an important factor in the transport of a variety of pollutants to the aquatic environment.

3 Organochlorine compounds from sources other than pesticides applications are involved in food webs and biological magnification in remote polar environments.

D "The data on water pollution, however, are less encouraging. Among other things, they indicate that land runoff from farms and even urban land, as opposed to discharges from cities and factories, has a much greater impact on water pollution than we realized. In all types of river basins, the concentration of nutrients, which can eutrophy our lakes, is increasing. These data indicate that while we carry on our major efforts to clean up pollution from municipal and industrial sources, we must increasingly turn our attention to land runoff-of nutrients, fertilizers, pesticides, organic materials, and the soil particles that often transport the others. If we fail to do so, our expenditures for water quality will not achieve maximum improvement." Council on Environmental Quality.

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This outline was prepared by Ralph M. Sinclair, Aquatic Biologist, National Training Center, WPO EPA, Cincinnati, OH 45268.

GLOBAL DETERIORATION AND OUR ENVIRONMENTAL CRISIS

I FROM LOCAL TO REGIONAL TO GLOBAL PROBLEMS

A Environmental problems do not stop at national frontiers, or ideological barriers. Pollution in the atmosphere and oceans taints all nations, even those benignly favored by geography, climate, or natural resources.

1 The smokestacks of one country can pollute the air and water of another.

2 Toxic effluents poured into an international river can kill fish in a neighboring nation and ultimately pollute international seas.

B In Antarctica, thousands of miles from pollution sources, penguins and fish contain DDT in their fat. Recent layers of snow and ice on the white continent contain measurable amounts of lead. The increase can be correlated with the earliest days of lead smelting and combustion of leaded gasolines.

C International cooperation, therefore, is necessary on many environmental fronts.

1 Sudden accidents that chaotically damage the environment - such as oil spills from a tanker at sea - require international cooperation both for prevention and for cleanup.

2 Environmental effects cannot be effectively treated by unilateral action.

3 The ocean can no longer be considered a dump.

D "One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise." Aldo Leopold

II CHANGES IN ECOSYSTEMS ARE OCCURRING CONTINUOUSLY

A Myriad interactions take place at every moment of the day as plants and animals respond to variations in their surroundings and to each other. Evolution has produced for each species, including man, a genetic composition that limits how far that species can go in adjusting to sudden changes in its surroundings. But within these limits the several thousand species in an ecosystem, or for that matter, the millions in the biosphere, continuously adjust to outside stimuli. Since interactions are so numerous, they form long chains of reactions.

B Small changes in one part of an ecosystem are likely to be felt and compensated for eventually throughout the system. Dramatic examples of change can be seen where man has altered the course of nature. It is vividly evident in his well-intentioned but poorly thought out tampering with river, lake, and other ecosystems.

1 The Aswan High Dam

2 The St. Lawrence Seaway

3 Lake Kariba

4 The Great Lakes

5 Valley of Mexico

6 California earthquake (Scientific American 3981, p. 333)

7 Everglades and the Miami, Florida Jetport

8 Copperhill, Tennessee (Copper Basin)

9 (You may add others)

C Ecosystem Stability

1 The stability of a particular ecosystem depends on its diversity. The more interdependencies in an ecosystem, the greater the chances that it will be able to compensate for changes imposed upon it.

2 A cornfield or lawn has little natural stability. If they are not constantly and carefully cultivated, they will not remain cornfields or lawns but will soon be overgrown with a wide variety of hardier plants constituting a more stable ecosystem.

3 The chemical elements that make up living systems also depend on complex, diverse sources to prevent cyclic shortages or oversupply.

4 Similar diversity is essential for the continued functioning of the cycle by which atmospheric nitrogen is made available to allow life to exist. This cycle depends on a wide variety of organisms, including soil bacteria and fungi, which are often destroyed by pesticides in the soil.

D Biological Pollution

Contamination of living native biotas by introduction of exotic life forms has been called biological pollution by Lachner et al. Some of these introductions are compared to contamination as severe as a dangerous chemical release. They also threaten to replace known wildlife resources with species of little or unknown value.

1 Tropical areas have especially been vulnerable. Florida is referred to as "a biological cesspool of introduced life".

2 Invertebrates

a Asian Clams have a pelagic veliger larvae, thus, a variety of hydro installations are vulnerable to subsequent pipe clogging by the adult clams.

b Melanian snails are intermediate hosts for various trematodes parasitic on man.

3 Vertebrates

a At least 25 exotic species of fish have been established in North America.

b Birds, including starlings and cattle egrets.

c Mammals, including nutria.

4 Aquatic plants

Over twenty common exotic species are growing wild in the United States. The problem of waterway clogging has been especially severe in parts of the Southeast.

5 Pathogens and Pests

Introduction of insect pests and tree pathogens have had severe economic effects.

III LAWS OF ECOLOGY

A Four principles have been enunciated by Dr. Barry Commoner.

1 Everything is connected to everything else.

2 Everything must go somewhere.

3 Nature knows best.

4 There is no such thing as a free lunch.

B These may be summarized by the principle, "you can't do just one thing."

IV THE THREE PRINCIPLES OF ENVIRONMENTAL CONTROL (Wolman)

A You can't escape.

B You have to organize.

C You have to pay.

V POLLUTION COMES IN MANY PACKAGES

A The sources of air, water, and land pollution are interrelated and often interchangeable.

1 A single source may pollute the air with smoke and chemicals, the land with solid wastes, and a river or lake with chemical and other wastes.

2 Control of air pollution may produce more solid wastes, which then pollute the land or water.

3 Control of wastewater effluent may convert it into solid wastes, which must be disposed of on land, or by combustion to the air.

4 Some pollutants - chemicals, radiation, pesticides - appear in all media.

B "Disposal" is as important and as costly as purification.

VI PERSISTENT CHEMICALS IN THE ENVIRONMENT

Increasingly complex manufacturing processes, coupled with rising industrialization, create greater amounts of exotic wastes potentially toxic to humans and aquatic life.

They may also be teratogenic (toxicants responsible for changes in the embryo with resulting birth defects, ex., thalidomide), mutagenic (insults which produce mutations, ex., radiation), or carcinogenic (insults which induce cancer, ex., benzopyrenes) in effect.

A Metals - current levels of cadmium, lead, and other substances whose effects on humans and fish and wildlife are not fully understood constitute a mounting concern. Mercury pollution, for example, has become a serious national problem, yet *mercury has been present* on earth since time immemorial. More research is needed, yet we dare not relax our standards until definitive answers have been provided.

B Pesticides

1 A pesticide and its metabolites may move through an ecosystem in many ways. Hard (pesticides which are persistent, having a long half-life in the environment includes the organo-chlorines, ex., DDT) pesticides ingested or otherwise borne by the target species will stay in the environment, possibly to be recycled or concentrated further through the natural action of food chains if the species is eaten. Most of the volume of pesticides do not reach their target at all.

2 Biological magnification

Initially, low levels of persistent pesticides in air, soil, and water may be concentrated at every step up the food chain. Minute aquatic organisms and scavengers, which screen water and bottom mud having pesticide levels of a few parts per billion, can accumulate levels measured in parts per million - a thousandfold increase. The sediments including fecal deposits are continuously recycled by the bottom animals.

a Oysters, for instance, will concentrate DDT 70,000 times higher in their tissues than it's concentration in surrounding water. They can also partially cleanse themselves in water free of DDT.

b Fish feeding on lower organisms build up concentrations in their visceral fat which may reach several thousand parts per million and levels in their edible flesh of hundreds of parts per million.

c Larger animals, such as fish-eating gulls and other birds, can further concentrate the chemicals. A survey on organochlorine residues in aquatic birds in the Canadian prairie provinces showed that California and ring-billed gulls were among the most contaminated. Since gulls breed in colonies, breeding population changes can be detected and related to levels of chemical contamination. Ecological research on colonial birds to monitor the effects of chemical pollution on the environment is useful.

C "Polychlorinated biphenyls" (PCB's). PCB's are used in plasticizers, asphalt, ink, paper, and a host of other products. Action has been taken to curtail their release to the environment, since their effects are similar to hard pesticides.

D Other compounds which are toxic and accumulate in the ecosystem:

1 Phalate esters - may interfere with pesticide analyses

2 Benzophyrenes

3

VII EXAMPLES OF SOME EARLY WARNING SIGNALS THAT HAVE BEEN DETECTED BUT FORGOTTEN, OR IGNORED.

A Magnetic micro-spherules in lake sediments now used to detect changes in industrialization indicate our slowness to recognize indicators of environmental change.

B Salmonid fish kills in poorly buffered clean lakes in Sweden. Over the past years there had been a successive increase of SO_2 in the air and precipitation. Thus, air-borne contamination from industrialized European countries had a great influence on previously unpolluted waters and their life.

C Minimata, Japan and mercury pollution.

D Organochlorine levels in commercial and sport fishing stocks, ex., the lower Mississippi River fish kills.

E You may complete the following

1

2

VIII SUMMARY

A Ecosystems of the world are linked together through biogeochemical cycles which are determined by patterns of transfer and concentrations of substances in the biosphere and surface rocks.

B Organisms determine or strongly influence chemical and physical characteristics of the atmosphere, soil, and waters.

C The inability of man to adequately predict or control his effects on the environment is indicated by his lack of knowledge concerning the net effect of atmospheric pollution on the earth's climate.

D Serious potential hazards for man which are all globally dispersed, are radionuclides, organic chemicals, pesticides, and combustion products.

E Environmental destruction is in lock-step with our population growth.

ACKNOWLEDGEMENT:

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CONVERSION FACTORS

Length

1 centimeter	0 394 inch
1 inch	2 540 centimeters
1 meter	3 2808 feet
1 foot	0 305 meter
1 meter	1 0936 yards
1 yard	0 9144 meter
1 kilometer	0 62137 mile
1 mile	1 60935 kilometers

Area

1 square centimeter	0 1550 square inch
1 square inch	6 452 square centimeters
1 square meter	10 764 square feet
1 square foot	0 09290 square meter
1 square meter	1 1960 square yards
1 square yard	0 8361 square meter
1 square kilometer	0 3861 square mile
1 square mile	2 590 square kilometers
1 acre (U S)	4840 square yards

Volume

1 cubic centimeter	0 0610 cubic inch
1 cubic inch	16 3872 cubic centimeters
1 cubic meter	35 314 cubic feet
1 cubic foot	0 02832 cubic meter
1 cubic meter	1 3079 cubic yards
1 cubic yard	0 7646 cubic meter

Capacity

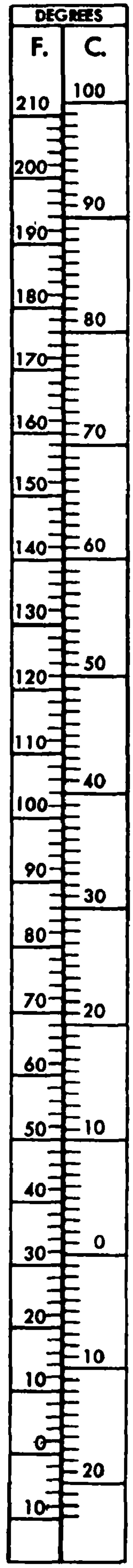
1 milliliter	0 03382 ounce (U S liquid)
1 ounce (U S liquid)	29 573 milliliters
1 milliliter	0 2705 dram (U S Apothecaries)
1 dram (U S Apothecaries)	3 6967 milliliters
1 liter	1 05671 quarts (U S liquid)
1 quart (U S liquid)	0 94633 liter
1 liter	0 26418 gallon (U S liquid)
1 gallon (U S liquid)	3 78533 liters

Mass

1 gram	15 4324 grains
1 grain	0 0648 gram
1 gram	0 03527 ounce (Avoirdupois)
1 ounce (Avoirdupois)	28 3495 grams
1 gram	0 03215 ounce (Troy)
1 ounce (Troy)	31 10348 grams
1 kilogram	2 20462 pounds (Avoirdupois)
1 pound (Avoirdupois)	0 45359 kilogram

Power

1 watt	0 73756 foot pound per second
1 foot pound per second	1 35582 watts
1 watt	0 056884 BTU per minute
1 BTU per minute	17 580 watts
1 watt	0 001341 Horsepower (U.S)
1 Horsepower (U S)	745 7 watts
1 watt	0 01433 kilogram-calorie per minute
1 kilogram-calorie per minute	69 767 watts
1 watt	1 x 10^7 ergs per second
1 lumen	0 001496 watt



SECTION H

BIOLOGICAL METHODS AND TECHNIQUES

The concept that use of water invariably results in changes in the quality or characteristics of the water was covered in the preceding section.

Methods then must be developed whereby a given water resource can be evaluated to determine its suitability for use and/or to determine the nature and extent of changes in the water that have occurred through use. Data interpretation demands a thorough knowledge of the conditions under which the samples were collected and a critical assessment of the reliability of the data's representation of the situation. Representative data sheets included here are not intended as a standard, but are designed to show how field observations may be integrated with subsequent laboratory analysis. Adequate field notes are essential for correct interpretation of laboratory data.

In this section consideration is given to a number of biological techniques for evaluation of water quality, and their application in action programs.

Contents of Section H

FUNDAMENTALS OF THE TOXICITY BIOASSAY

I INTRODUCTION

A The toxicity bioassay procedure herein discussed is intended for use by industrial and other laboratories.

B Its objective is to evaluate the toxicity of wastes and other water pollutants to fish or other aquatic organisms.

C This basic procedure evaluates relatively acute toxicity only (chronic or cumulative toxicity requires more extensive study).

D Potential applications are numerous.

1 Dilution and/or treatment necessary to avoid acute toxic effects can be estimated.

2 The efficacy of a treatment can be tested.

3 The potential usefulness of a proposed treatment can be estimated.

E The toxicity bioassay technique does not involve a chemical knowledge of the toxicant.

1 Synergism, antagonism, and other interactions of chemical components cannot always be anticipated, but are automatically included in the overall evaluation.

2 All chemical and physical information available is essential to the adequate interpretation and application of test results.

F The test is designed for local application. Generalizations should be made with great caution.

G Field observations should be made of results of application over a significant period of time.

H Careful distinction should be made between fish mortality due to a physiological toxicant, and that due to lack of DO.

I A uniform testing procedure is essential to effective action in water pollution control.

II ROUTINE PROCEDURE

A <u>Test animals</u> should be fish or other organisms of local significance.

1 Extremely resistant or extremely sensitive species should not be selected.

2 They should be species which are amenable to captivity.

3 They should be accurately identified.

4 They should be relatively uniform in size. Individuals less than 3 inches in length are usually most convenient.

5 They should be healthy and thoroughly acclimated to the laboratory.

B <u>Test water</u> should preferably be taken from the receiving stream just above the discharge being evaluated.

1 If this is unsuitable, cleaner waters from an upstream station may be substituted.

2 Artificial "standard" waters are not recommended for general use.

C Other Experimental Conditions

1 Temperature

The tests should be performed at a uniform temperature in the upper part of the expected summer range, e.g., 20° - 25° C for warm water fish, and 12° - 18° C for cold water species. It has been found however, that for most routine operations, ambient laboratory temperatures are satisfactory. Standard modern air conditioning, particularly if it is maintained 24 hours a day, is quite adequate.

2 Test containers should be of glass. Wide mouthed "pickle jugs" or battery jars are satisfactory. Five and one gallon sizes are both useful, but the larger size is required for conclusive results.

3 Artificial Aeration should not be used to maintain the dissolved oxygen concentration. If this falls below approximately 4 or 5 ppm at any time during the test, fewer fish should be used per container or an auxiliary oxygenation procedure invoked that is designed to void undue loss of volatile toxicants.

4 The number of test animals should not be less than 10 per concentration for reliable conclusions, these may be distributed between two or more containers.

5 Ratio of fish to solution

There should be less than 2 grams of fish per liter of test solution, preferably not more than one.

D Experimental Procedure

1 All dilutions for a given run should be prepared from the same sample.

2 Control tests are essential.

3 Duration

Tests should be run for at least 48 hours, preferably 96.

4 Dead fish should be removed as soon as observed. Survivors should be counted and recorded each 24 hours.

5 Feeding during the test should be avoided.

6 Experimental concentrations

Any appropriate concentrations may be used. A logarithmic series such as is suggested in Table I is very convenient. Concentrations can be expressed in percent by volume, parts per million by weight, or other appropriate units.

7 Expression of results

The measure of relative toxicity is the median tolerance limit (symbol: TL_m, this is the analogue of the LD_{50} of the toxicologist).

a This is the concentration which just 50% of the test animals can survive for a stipulated period of time (sometimes written TL^t_m where t = 24, 48, 96 hours, etc.)

b The TL^t_m may conveniently be estimated graphically, by plotting the experimental data on semi-logarithmic graph paper, with the test concentration laid off on the log, scale, and the percent survival on the arithmetic scale. Connect with a straight line the two successive data points representing survival values of greater than and less than 50%. Note the concentration which corresponds to 50% survival on this graph. This is the "TL^t_m". Other methods are acceptable.

III REPORTING, INTERPRETATION AND APPLICATION

A Reports should include an orderly tabulation of all pertinent data such as:

1 Identity of experimental animals

2 Their source, average size and condition, and number used per concentration

3 Source and chemical and physical analysis of experimental water

4 Experimental temperature

5 Volumes of experimental liquid in each container

6 Records of running analyses such as DO and pH

7 TL_m and data from which it was determined

B Interpretation and application will be discussed more thoroughly later. Briefly

1 The TL_m is an estimate of the midpoint of the critical concentration range (the interval between the highest concentration at which all test animals survive, and the lowest at which they all die).

2 The problem is to extrapolate from this well established mid concentration to a safe concentration well below the "critical concentration range".

3 Initiation of regulatory procedures based on the TL_m should be followed by periodic field observations. If aquatic life flourishes, there is no problem indicated. If not, the material must be still further diluted.

IV SPECIAL PROBLEMS

A Unaerated aquaria with finite quantities of toxicant are not always satisfactory (Static Tests).

1 The toxicant may be volatile.

2 Toxic materials may be masked by a high BOD.

3 The toxicant may be progressively adsorbed or otherwise changed.

TABLE I

A Guide to the Selection of Experimental Concentrations, Based on Progressive Bisection of Intervals on a Logarithmic Scale.

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0	----	----	----	----
----	----	----	----	8.7
----	----	----	7.5	----
----	----	----	----	6.5
----	----	5.6	----	----
----	----	----	----	4.9
----	----	----	4.2	----
----	----	----	----	3.7
----	3.2	----	----	----
----	----	----	----	2.8
----	----	----	2.4	----
----	----	----	----	2.1
----	----	1.8	----	----
----	----	----	----	1.55
----	----	----	1.35	----
----	----	----	----	1.15
1.0	----	----	----	----

B Standards or requirements other than those involving toxicity per se may be involved.

C Preliminary and Concurrent Investigations

1 Obtain all available information about unknown to be tested.

2 Does the material lend itself to this type of test?

3 Run feasible on the spot analyses including DO.

4 Significant quantities of solutions removed from test containers for analysis should be replaced with similar volume of same dilution.

D Wastes with a high BOD or COD

1 Suggested preliminary tests

a Set up two identical exploratory tests.

b Aerate one but not the other.

c If great difference develops between them, special procedures are indicated.

2 Oxygenation or aeration of dilution water before making dilutions may help.

3 Oxygenation of experimental containers during run. Pure oxygen is suggested instead of air in order to avoid the bubbling any more gas through the containers than is necessary as some of the toxic fraction may be volatile materials which would be stripped out.

a Lead oxygen into tank through glass tube instead of breaker stone in order to keep bubbles large.

b Control rate of bubbling. Keep it at the minimum number of bubbles per minute which will maintain 4 to 5 milligrams of oxygen per liter. Do not attempt saturation.

c Other systems of oxygenation are available.

4 Renewal of solutions at stated intervals (12, 24, or 48 hours) is approved. Fish are not harmed by being carefully transferred from one container to another. It is useful where·

a Initial DO is adequate but slowly exhausted.

b Toxicant is volatile, progressively adsorbed, precipitated, or otherwise changed.

E Continuous flow apparatus is highly desirable but expensive.

1 Equipment more involved and subject to failure during a run.

2 May be adapted to monitoring by use of proportioning equipment. Makes longer runs possible.

F Other Considerations

1 Radioactive wastes must be evaluated in regard to their chemical toxicity as well as their radioactivity.

2 Sub acute levels of many toxicants such as lead, arsenic, chromium, etc., may exert a low level chronic toxicity over a long period of time.

3 "Safe levels" of a waste in regard to toxicity may still exceed standards of other types such as color, organic content, suspended solids, etc.

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BIOMONITORING OF INDUSTRIAL EFFLUENTS

I INTRODUCTION

A Plant operating personnel need to know the general quality of an effluent which is being discharged at a fairly constant rate and also must be warned if a slug of toxic material is released to the receiving water.

B Conventional bioassay procedures can evaluate only single samples taken at particular times. Continuous-flow bioassays of single grab samples over a long period of time can be very useful, but do not solve the problem of transient variations.

C A technique that does permit exercising continuous surveillance over the toxicity of an effluent is biomonitoring

D Conventional bioassays [2, 3, 4] can provide important information about the actual toxicity of batches of the effluent in terms of TL_m's, and, if sufficient samples are tested, about the range of variation. Bioassays should be run from time to time to ascertain the exact toxicity of a waste even though it is being monitored as outlined below.

E The following procedures refer only to toxic wastes having a relatively rapid action. Wastes such as cadmium which have long delayed cumulative effects at low concentrations, [5] oxygen-demanding wastes, radioactive wastes, and others, would either be inappropriate or would not elicit a recognizable reaction soon enough to be of use.

II OBJECTIVES OF BIOMONITORING

Three basic objectives of biomonitoring are:

A To demonstrate the continuous suitability of an effluent for aquatic life provided slow-acting or cumulative toxins are not involved. The continuous testing of an undiluted effluent, (Objective A) is usually accomplished by leading a small stream of the effluent through an aquarium. This aquarium may be located in a public lobby to enhance public relations, or it may be in the plant for operational use only. This is a relatively simple and direct approach and needs no further elaboration.

B To detect change (usually deleterious) in the biological acceptability of the effluent itself.

C To detect change in the effect of the effluent on the biota of the receiving water.

III EQUIPMENT

A single basic design of exposure tanks and flow plan can be used to accomplish Objective B or C. (Figure 1) With the exception of a simple suggestion for proportioning flow of effluent to dilution water (Figure 2), engineering devices for accomplishing the various needs outlined below are not discussed. [6, 7, 8] Special care should be used to ensure that all surfaces that come in contact with the waste or the dilution water are constructed of nontoxic and noncorrosive materials. This precaution is particularly necessary for marine waters, where bimetallic contacts are very dangerous. An experienced aquatic biologist should be consulted in the preparation of plans.

A Exposure Tanks

1 Exposure tanks (Figure 1) should be large enough (10 to 20 gallons) that the test organisms can live normally under plant conditions. [3] Simple construction

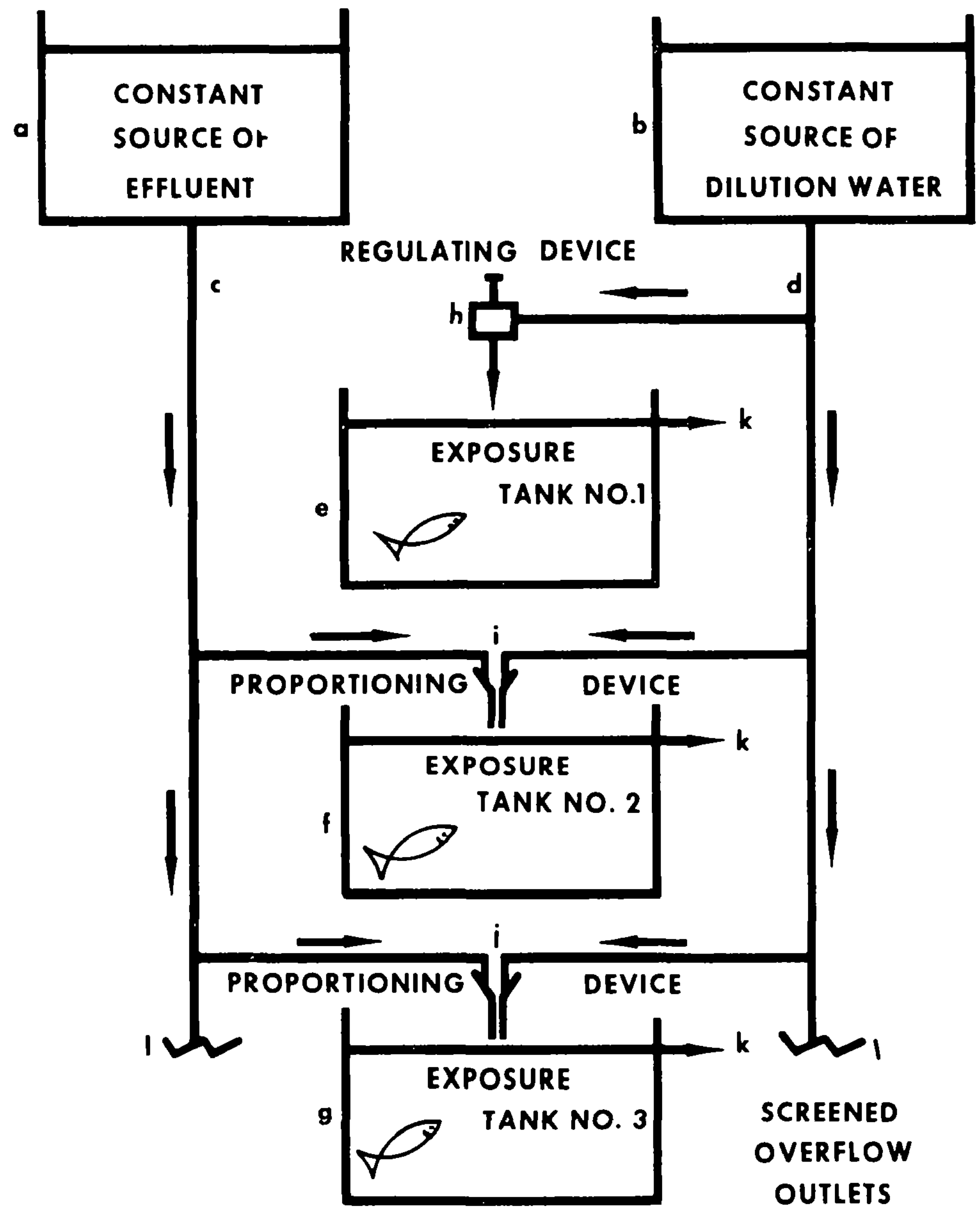

Figure 1. SCHEMATIC FLOW PLAN FOR OBJECTIVES B OR C

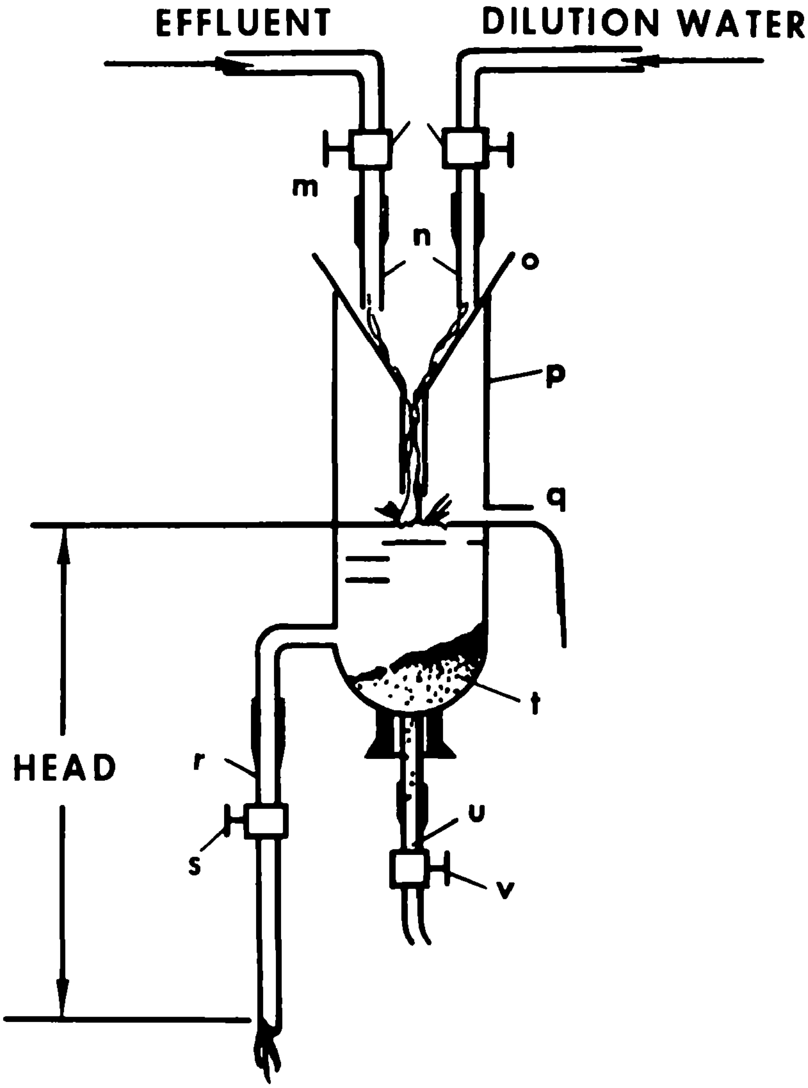

Figure 2

SUGGESTED PLAN FOR PROPORTIONING FLOW BETWEEN EFFLUENT AND DILUTION WATER. (See i and j, Figure 1) will facilitate feeding, cleaning, and disease control. "Eye-appeal" is not necessary unless public relation is involved, but scrupulous sanitation is essential as in all long-term animal culture.[9, 10] Tanks should be situated in a lighted and well-ventilated room, but not exposed to direct sunlight. Ambient room temperatures are generally satisfactory.

2 Inlet and overflow should be similar in all tanks so that conditions will be the same except for the quality of the water. The total flow of water through the tank should be adjusted so that the hydraulic retention times are equal, whether the total flow is coming from one source or two.

3 Tank No. 1 contains unadulterated dilution water to establish that the test animals will live in it. This tank is the control or "reference" to which the other tanks are compared.

4 Tanks 2 and 3 (more may be added at points 1) contain mixtures of effluent and dilution water. If the experimental animals die or show distress in these tanks, a change for the worse in the characteristics of the effluent being monitored is probably indicated.

5 i and j are mixing or proportioning devices set to predetermined amounts.

6 All tanks overflow to the sewer through opening k, which should be screened to prevent escape of test animals and clogging in outlet pipe.

B Effluent Supply

The supply of effluent should be constant and controllable. The prime requisite is that it be fresh so that changes may be detected at the earliest possible moment.

C Dilution Water

1 The source and quality of the dilution water is the most important single factor in the system because this is the scale or standard by which the toxicity of the effluent is assayed.

2 To test the effect of the effluent on the receiving water (Objective C), the dilution water obviously must be the receiving water.

a In most cases the receiving water varies from time to time as runoff water washes in different materials from the surface of the land the tide turns, or other plants release various wastes.

b Plan C (Objective C) automatically evaluates the toxicity of the effluent when discharged into this changing situation. This water should contain all of the components present down to the outfall being monitored, but none of the effluent itself.

3 In a stream situation, dilution water can be taken well upstream from the waste discharge. A pipeline with a continuous flow is ideal, since slugs of material from upstream sources that might modify the toxicity of the effluent being monitored would be taken into the monitoring system and quickly distributed to the exposure tanks. Because such systems are notoriously subject to "problems," batch transportation of control water may have to be employed. In lakes, estuarine, or coastal situations, batch supply may be the only practical solution.

4 If the receiving water is already continuously toxic to aquatic life, it is unsuitable for use in the system.

a Under such circumstances Plan C would be unworkable and the only recourse would be Plan A or B.

b Treated water such as drinking water from a city or industrial plant should never be used for any of these plans, even if dechlorinated, because the chemicals used in treatment may react with the waste being monitored.

D Proportioning Flow

The plan (Figure 2) by which effluent and dilution water are proportioned to the test tanks (Tank 2 and 3) determines what the system will accomplish.

1 A suggested plan for proportioning flow between effluent and dilution water (i and j) in Figure 1 is given in Figure 2.

2 Rubber tubes (n) can be momentarily diverted to catch flow in an appropriate sampling device such as a graduated cylinder. Based on the time required to discharge some standard volume, flow could be proportioned to any desired ratio (for example, one part effluent to two parts dilution water).

3 The removal of sediment by some sort of trap as shown is a policy matter that should be resolved in the project-design statement.

IV FLOW PLANS

A Flow Plan for Objective B

If the system is to be operated to detect only serious detrimental change in the effluent itself, Tanks 2 and 3 should contain mixtures in such proportions of effluent and dilution water as to permit the test animals to live as long as the effluent is normal.

1 One possible combination would be to adjust the mixing mechanism at i (Figure 1) to admit such a proportion of effluent that the test animals in Tank No. 1 could barely survive. The slightest increase in toxicity of the effluent would then immediately be made evident by the death of the test animals and appropriate remedial action could be taken.

2 The mixing mechanism j in Tank No. 3 might be adjusted to provide a greater margin of safety, for example 1/2 or 1/10th the toxicity of Tank 2. If Tank 2 animals then died, but Tank 3 animals survived, it would presumably indicate only a moderate increase in toxicity.

B Flow Plan for Objective C

Monitoring the effect of the effluent on the receiving water, from the point of view of protecting aquatic life, is ideal, but may also be very difficult.

1 The reference tank (e, Figure 1) receives water fresh from the receiving body. This water is free of any trace of the effluent being monitored, but contains all substances, natural and artificial, presently in the receiving water. These components may change from time to time, and one of these changes may increase the toxic effect of the effluent (synergism).

Thus the death of animals in the strongest test tank, but not in the reference tank, may be the result of an increase in the toxicity of the plant waste, or of a synergistic reaction of the effluent with a material in the receiving water.

2 No matter the cause, the death itself serves as a warning and immediate action can be taken on the discharge to protect aquatic life.

3 On the other hand, if a slug of strongly toxic matter enters the receiving water from some outside source, the deaths of the animals in the reference Tank 1 (and probably also those in exposure Tanks 2 and 3) would show that the "fish kill" presumably in progress in nature was not the result of the effluent.

4 A parallel installation under Plan B might also demonstrate no change in the waste being monitored.

5 Numerous other dilution systems might also be employed under Plan C.

C A different approach is to proportion the mixture to simulate the actual mixture taking place in the receiving water. For this purpose if the location were on a flowing stream, the flow of both the stream and final effluent must be known. Periodic adjustments might be made by hand, or by automatic equipment involving telemetry of both effluent and stream flow.

V TEST ANIMALS AND OTHER CONSIDERATIONS

No universal recommendations can be made about test animals to be used although many suggestions are available.[1, 3, 5]

A Irwin[11] investigated the suitability of 57 species of freshwater fishes for this purpose. Briefly, they should be of local importance, they must be a type that can be maintained in good health in the laboratory in the dilution water to be used, and enough must be employed so that reasonable statistical reliability is assured (for example, 10 per tank).

1 Fish are usually employed as test animals, although there is no reason not to use any other organisms that can be successfully kept alive in the test tanks.[12]

2 Continuous availability is also important. It is well not to change the test species after a program has been established, since the reactions of different species to the waste being monitored may not be the same.[11]

3 Animals in the exposure tanks should be fed the same as those in the stock tanks. The same kind of food in the same ratio of food to weight of test fish should be added to each tank at the same intervals. Unnatural acceptance of food in exposure tank may indicate a measure of distress, even in the absence of mortality.

B Oxygen determinations should be run occasionally to ensure that any deleterious symptoms are the result of the effluent and not of oxygen deficiency. Actual minimum acceptable levels will depend on the temperature and type of fish used.

C Long-continued exposure to low level concentrations in tanks may result in cumulative intoxication, or acclimatization.

1 In most cases these effects can probably be best counteracted by periodic renewal of the test fishes, for example at 60-day intervals.

2 When obtaining stocks of test animals from receiving waters, these same factors should be borne in mind. Fish or other organisms taken from below the outfall might have acquired some immunity or sensitivity to the effluent being tested.

3 Fish or a species normally present in the receiving stream, but imported from some other (unpolluted) source, will presumably exhibit a completely unconditioned response. Generally, since the aquatic life to be protected is that already present in the stream, the most logical source of fish is the stream itself. Under operating conditions, however, it is not always practicable to collect the experimental animals from this source and imports from another area may be necessary.

D Selection of Dilutions

1 The "critical range" of toxicity may be defined as the range between the highest concentration that kills no test animals and the lowest concentration that kills all. The TL_m of the conventional bioassay[3] is in the middle portion of this range.

2 When an effluent is to be biomonitored under Plan B, the selection of appropriate dilutions might be based on the above concept of "critical range."

a If a "tight" control is desired, the highest concentration might be established near the TL_m. When a batch of test animals is first placed in such a dilution, approximately half of them may (by design) be expected to die. The survivors, however, would constitute a rigorous control as any increase in toxicity would be expected to kill the remaining animals in order of susceptibility, until, as the top of the critical range is reached, all would be dead.

b A somewhat less stringent control would be effected with a dilution near the lower end of the critical range.

3 In any large population of test animals kept in an exposure tank over an extended period of time, an occasional animal may be expected to die.

a The mortality of significance then is not the occasional individual death, but the sudden death of 25, 50, or 100% of the test animals.

b When this happens, biomonitoring has sounded the alarm to take appropriate action to detoxify the effluent or to divert it from the receiving stream until it is again normal.

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BIOLOGICAL FIELD METHODS

I INTRODUCTION

A Due to the nature of ecological interrelationships, methods for the collection of different types of aquatic organisms differ. In general we can recognize those that swim or float and those that crawl, those that are big and those that are little. Each comprises a part of "the life" at any given survey station and consequently a "complete" collection would include all types.

B Field methods in the following outline are grouped under four general categories, the collection of·

1 Benthos (or bottom dwelling organisms). These may be attached, crawling, or burrowing forms.

2 Plankton (plancton). These are all of the microscopic plants and animals normally swimming or suspended in the open water

3 Periphyton or "aufwuchs". This is the community of organisms associated with the surfaces of objects. Some are attached, some crawl. The group is intermediate between the benthos and the plankton.

4 Nekton. Nekton are the larger, free swimming active animals such as shrimp or fishes.

C Aquatic mammals and birds, in most cases, require still other approaches and are not included.

D There is little basic difference between biological methods for oceanic, estuarine, or freshwater situations except those dictated by the physical nature of the environments and the relative sizes of the organisms. Fish, benthos, and plankton collection is essentially the same whether conducted in Lake Michigan, Jones' Beach, or the Sargasso Sea.

1 Marine organisms range to larger sizes, and the corrosive nature of seawater dictates special care in the design and maintenance of marine equipment. Site selection and collection schedules are influenced by such factors as tidal currents and periodicity, and salinity distribution, rather than (river) currents, riffles, and pools.

2 Freshwater organisms are in general smaller, and the water is seldom chemically corrosive on equipment. Site selection in streams involves riffles, falls, pools, etc., and a unidirectional flow pattern. Lake collection may involve less predictable stratification or flow patterns.

E Definite objectives should be established in advance as to the size range of organisms to be collected and counted, i.e. microscopic only, microscopic and macroscopic, those retained by "30 mesh" screens, invertebrates and/or vertebrates, etc.

II STANDARD PROCEDURES

A Certain standard supplementary procedures are a part of all field techniques. In order to be interpreted and used, every collection must be associated with a record of environmental conditions at the time of collection.

1 Data recorded should include the following as far as practicable.

Location (name of river, lake, etc.)

Station number (particular location of which a full description should be on record)

Date and hour

Air temperature

Water temperature (at various depths, if applicable)

Salinity (at various depths, if applicable)

Tidal flow (ebb or flood)

Turbidity (or light penetration, etc.)

Weather

Wind direction and velocity

Sky or cloud cover

Water color

Depth

Type of bottom

Type of collecting device and accessories

Method of collecting

Type of sample (quantitative or qualitative)

Number of samples at each station

Chemical and physical data, e.g., dissolved oxygen, nutrients, pH, etc.

Collector's name

Miscellaneous observations (often very important)

2 All collecting containers should be identified at least with location, station number, sample number, and date. Spares are very handy.

3 Much transcription of data can be eliminated by using sheets or cards with a uniform arrangement for including the above data. The same field data sheet may include field or laboratory analysis.

B Compact kits of field collecting equipment and materials greatly increase collecting efficiency, especially if collection site is remote from transportation.

III PERSONAL OBSERVATION AND PHOTOGRAPHY

A Direct or indirect observation of underwater conditions has become relatively efficient.

1 Diving spheres, pioneered by William Beebe, Cousteau, Honot, Willm, and Manad are proving very important for deep water observations.

2 Use of the aqualung permits direct personal study down to over 200 feet.

3 Underwater television (introduced by the British Admiralty for military purposes) is now generally available for biological and other observations.

4 Underwater photography is improving in quality and facility.

5 Underwater swimming or use of SCUBA is quite valuable for direct observation and collecting.

IV COLLECTION OF BOTTOM OR BENTHIC ORGANISMS

A Shoreline or Wading Depth Collecting Plates I, II

1 Hand picking of small forms attached to or crawling on rocks, sticks, etc. when lifted out of the

BOTTOM GRABS

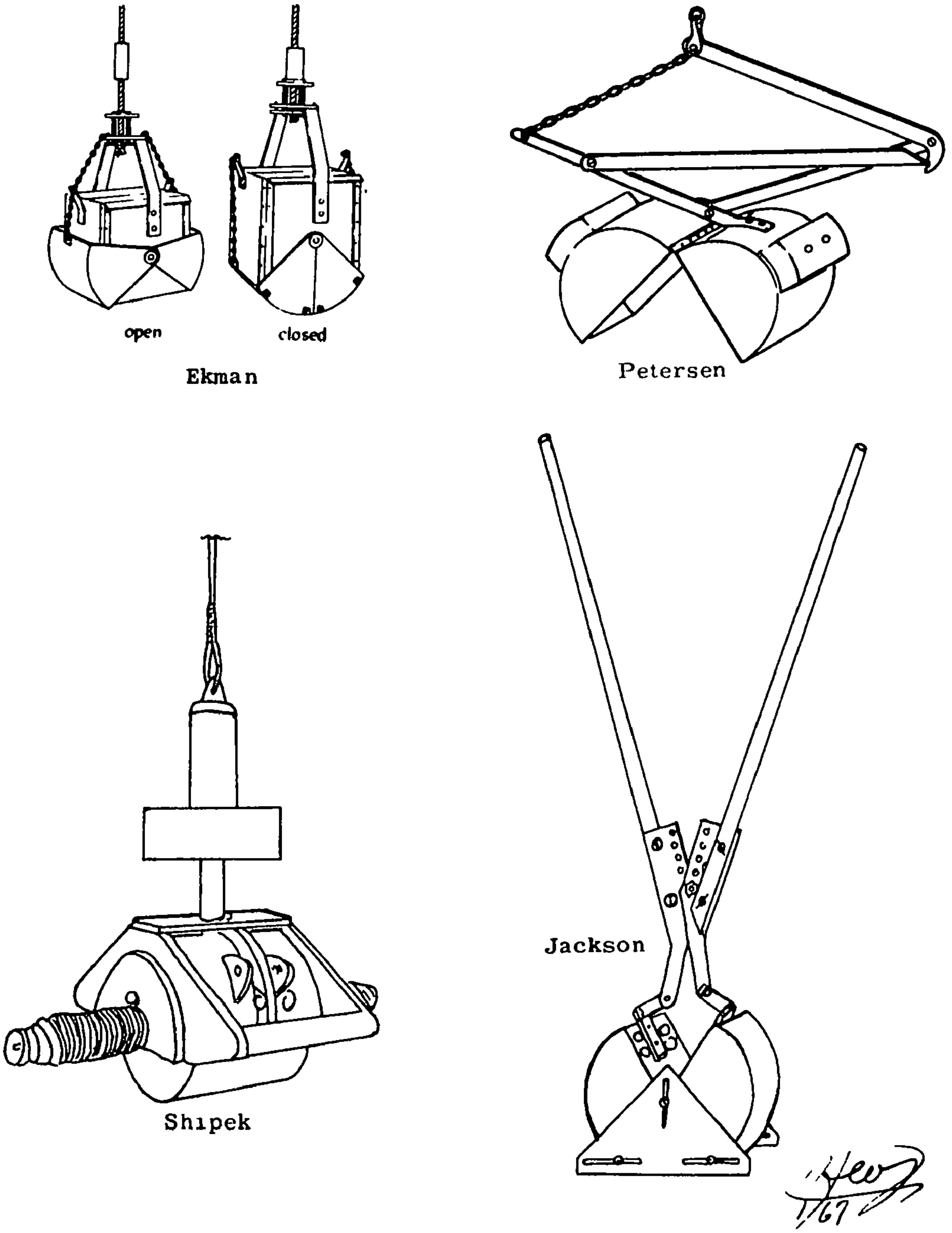


PLATE I

LIMNOLOGICAL EQUIPMENT

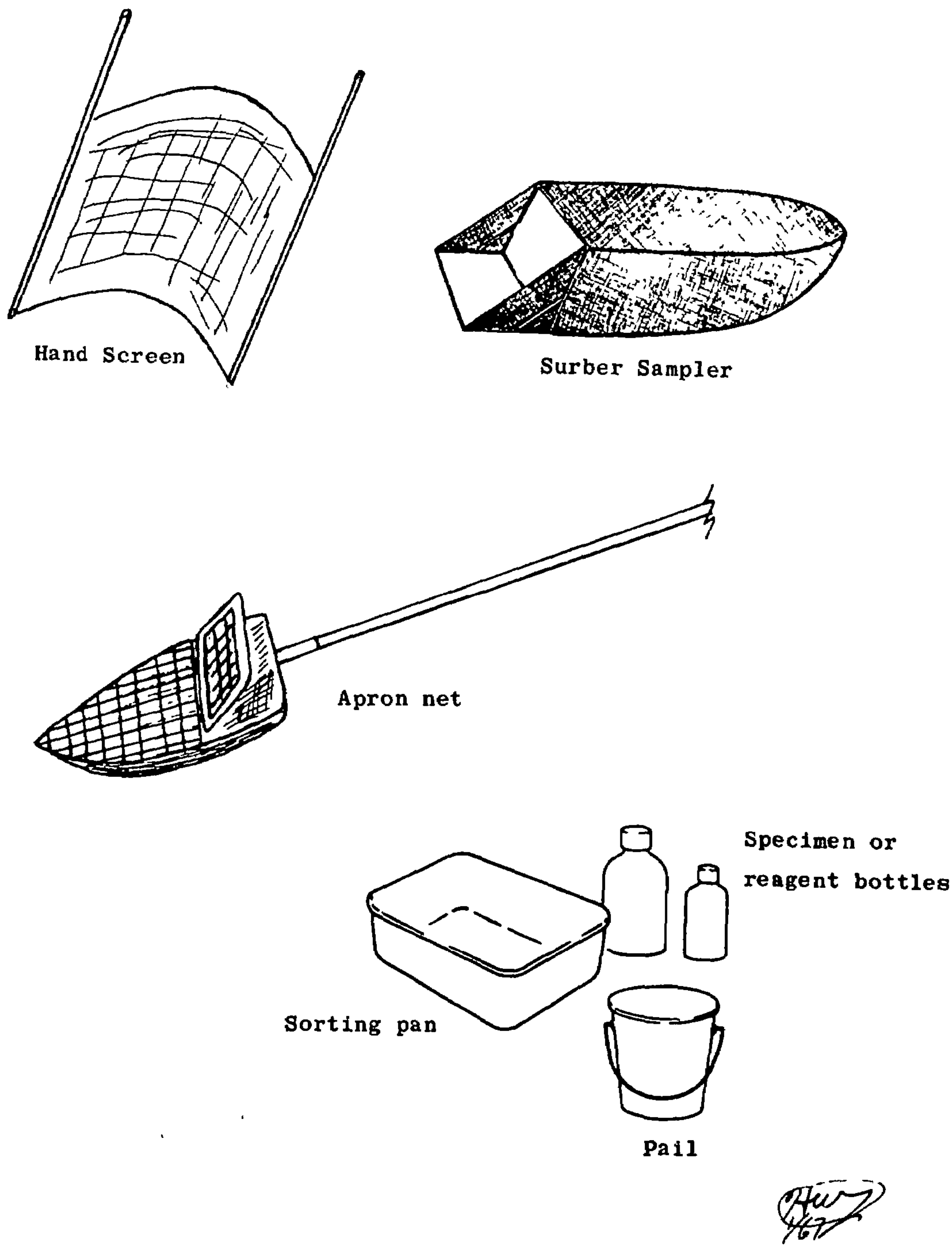


PLATE II

water is a fundamental and much used method for quickly assaying what is present and what may be expected on further search.

2 Patches of seaweed and eelgrass and shallow weedy margins anywhere are usually studied on a qualitative basis only.

a The apron net is one of the best tools for animals in weed beds or other heavy vegetation. It is essentially a pointed wire sieve on a long handle with coarse screening over the top to keep out leaves and sticks.

b Grapple hooks or a rake may be used to pull masses of vegetation out on the bank where the fauna may be examined and collected as they crawl out.

c Quantitative estimates of both plants and animals can be made with a "stove pipe" sampler which is forced down through a weed mass in shallow water and embedded in the bottom. Entire contents can then be bailed out into a sieve and sorted.

d A frame of known dimensions may be placed over an area to be sampled and the material within cropped out. This is especially good for larger plants and large bivalves This method yields quantitative data.

3 Sand and mud flats in estuaries and shallow lakes may be sampled quantitatively by marking off a desired area and either digging away surrounding material or excavating the desired material to a measured depth. Handle-operated samplers recently developed by Jackson and Larrimore, make for more effective sampling of a variety of bottoms down to the depth of the handles. Such samples are then washed through graded screens to retrieve the organisms.

4 Ekman grabs are most useful on soft bottoms. This is a completely closing clamshell type grab with spring operated jaws. Size of grab is usually 6" X 6" or 9" X 9", the 12" X 12" size is impractical due to its heavy weight when filled with bottom material.

For use in shallow water, it is convenient to rig an Ekman with a handle and a hand operated jaw-release mechanism.

5 The Petersen type grab (described below) without weights will take satisfactory samples in firm muds, but tends to bury itself in very soft bottoms. It is seldom used in shallow water except as noted below.

B Collecting in Freshwater Riffles or Rapids

1 The riffle is one of the most satisfactory habitats for comparing stream conditions at different points.

2 The hand screen is the simplest and easiest device to use in this situation. Resulting collections are qualitative only.

a In use the screen is firmly planted in the stream bed. Upstream bottom is thoroughly disturbed with the feet, or worked over by hand by another person. Organisms dislodged are carried down into the screen.

b Screen is then lifted and dumped into sorting tray or collecting jar.

3 The well-known square foot Surber sampler is one of the best quantitative collecting devices for riffles.

a It consists of a frame one foot square with a conical net attached. It is usable only in moving water.

b In use it is firmly planted on the bottom. The bottom stones and gravel within the square frame are then carefully gone over by hand to ensure that all organisms have been dislodged and carried by the current into the net. A stiff vegetable brush is often useful in this regard.

c From three to five square-foot samples should be taken at each station to insure that a reasonable percentage of the species present will be represented.

4 The Petersen type grab may be used in deep swift riffles or where the Surber is unsuitable.

a It is planted by hand on the bottom, and worked down into the bottom with the feet.

b It is then closed and lifted by pulling on the rope in the usual manner.

5 A strong medium weight dipnet is the closest approach to a universal collecting tool (effective for everything from Blondes to indicator organisms!)

a This is used with a sweeping motion, through weeds, over the bottoms or in open water. A triangular shape is preferred by some. This may be used as a roughly quantitative device in riffles by holding the end flat against the bottom and backing slowly up-stream disturbing the substrate with one's feet. A standard period of time is used.

b The handle should be from 4 to 6 feet long, and about the weight of a garden rake handle.

c The ring should be made of steel or spring brass, and securely fastened to the handle. It should be strong but not cumbersome; size of ring stock will depend on diameter of ring.

d The bag or net should be the strongest available, not over 1/8 inch mesh, preferably about 1/16 inch. Avoid 30 or more meshes to the inch, this is so fine that the net plugs too easily and is slow and heavy to handle.

e There should be a wide canvas apron sewed around the rim and protecting the bag. The rim may be protected with leather if desired.

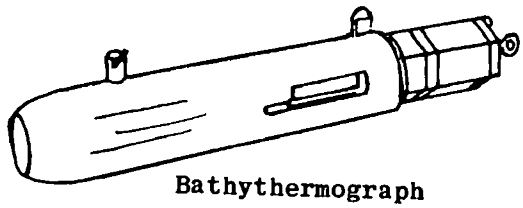
D Deep Water Benthic Collecting Plate III

1 When sampling from vessels, a crane and winch, either hand or power operated, is used. The general ideas described for shallow waters apply also to deeper waters, when practicable.

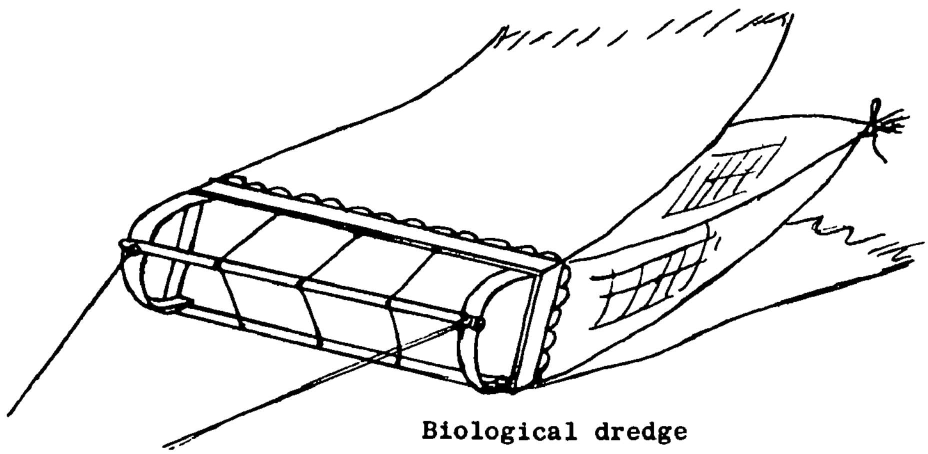
2 The Petersen type grab, seems to be the best all around sampler for the greatest variety of bottoms at all depths, from shoreline down to over 10, 000 meters. (Plate I)

a It consists of two heavily constructed half cylinders closed together by a strong lever action.

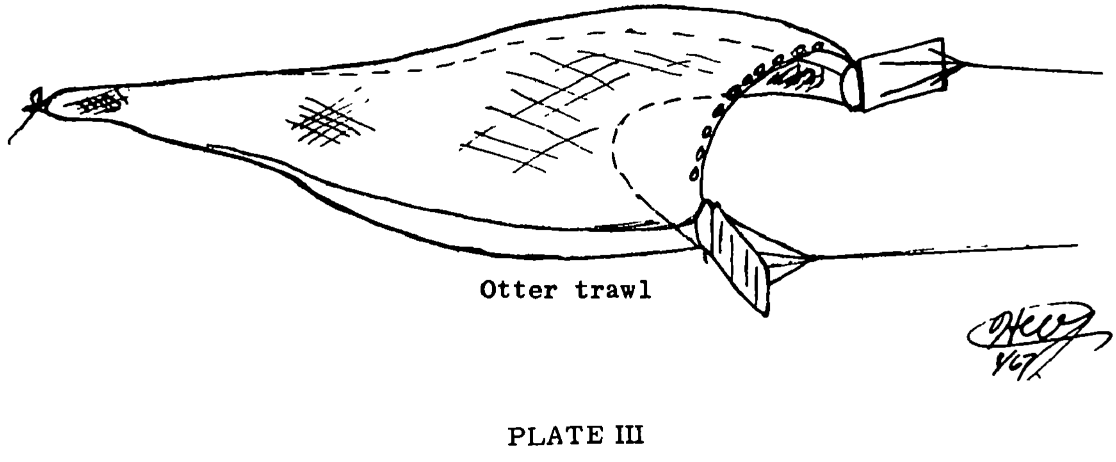
DEEP WATER EQUIPMENT



Bathythermograph



Biological dredge



Otter trawl

PLATE III

b To enable them to bite into hard bottoms, or to be used in strong currents, weights may be attached to bring the total weight up to between 50 and 100 lbs.

c Areas sampled range from 1/5th to 1/20th square meters (1/10 square meter equals approximately 1.1 square ft.)

d A Petersen grab to be hauled by hand should be fitted with 5/8 or 3/4 inch diameter twisted rope in order to provide adequate hand grip. It is best handled by means of wire ropes and a winch.

3 Other bottom samplers include the VanVeen, Lee, Holme, Smith-McIntyre, Knudsen, Ponar, and others.

4 A spring loaded sampler has recently been developed by Shipek for use on all types of bottoms. It takes a half-cylinder sample, 1/25th square meters in area and approximately 4 inches deep at the center. The device is automatically triggered on contact with the bottom, and the sample is completely protected enroute to the surface. (Plate I)

5 Drag dredges or scrapes are often used in marine waters and deeper lakes and streams, and comprise the basic equipment of several types of commercial fisheries. Some types have been developed for shallow streams. In general however, they have been little used in fresh water.

6 The above is only a partial listing of the many sampling devices available. Others that are often encountered are the orange-peel bucket, plow dredge, scallop type dredge, hydraulic dredges, and various coring devices. Each has its own advantages and disadvantages and it is up to the worker and his operation to decide what is best for his particular needs. The Petersen type and Ekman grabs are perhaps the most commonly used.

7 Traps of many types are used for various benthic organisms, especially crabs and lobsters. Artificial substrates (below) are in essence a type of trap.

8 Since most biological communities are not evenly distributed, it is advisable to routinely take at least two and preferably more samples from any one station.

E Artificial substrates rely on the ecological predilection of organisms to grow wherever they find a suitable habitat. When a small portion of artificial habitat is provided, it tends to become populated by all available species partial to that type of situation. The collector can then at will remove the habitat or trap to his laboratory and study the population at leisure.

This versatile research technique is much used for both routine monitoring and exploratory studies of pollution. It is also exploited commercially, especially for shellfish production. Types of materials used include.

1 Cement plates and panels.

2 Wood (especially for burrowing forms).

3 Glass slides (ex Catherwood diatometer).

4 Multiple plate trap (masonite).

5 Baskets (or other containers) holding natural bottom material and either imbedded in the bottom, or suspended in the overlying water.

6 Unadorned ropes suspended in the water, or sticks thrust into the bottom.

F Sorting and Preservation of Collections

1 Benthic collections usually consist of a great mass of mud and other debris among which the organisms are hidden. Various procedures may be followed to separate the organisms.

a The organisms may be picked out on the spot by hand or the entire mess taken into the laboratory where it can be examined more efficiently (especially in rough weather). Roughly equivalent time will probably be required in either case.

b Specimens may be simply observed and recorded or they may be preserved as a permanent record.

c Organisms may be simply counted, weighed, or measured volumetrically; or they may be separated and recorded in groups or species.

2 If separation is in the field, this is usually done by hand picking, screening, or some type of flotation process.

a Hand picking is best done on a white enameled tray using light touch limnological forceps.

b Screening is one of the most practical methods to separate organisms from debris in the field. Some prefer to use a single fine screen, others prefer a series of 2 or 3 screens of graded sizes. The collection may be dumped directly on the screen and the mud and debris washed through, or it may be dumped into a bucket or small tub. Water is then added, the mixture is well stirred, and the supernatant poured through the screen. The residue is then examined for heavy forms that will not float up.

c A variation of this method in situations where there is no mud is to pour a strong sugar or salt solution over the collection in the bucket, stir it well, and again pour the supernatant through the screen. This time, however, saving the flotation solution for re-use. The heavier-than-water solution accentuates the separation of organisms from the debris (except for the heavy shelled molluscs, etc.). A solution of 2-1/2 lbs. of sugar per gallon of water is considered to be optimum.

3 Preservation or stabilization is usually necessary in the field.

a 95% ethanol (ethyl alcohol) is highly satisfactory. A final strength of 70% is necessary for prolonged storage. If the collection is drained of water and flooded with 95% ethanol in the field, a laboratory flotation separation can usually be made later, thus saving much time. Considerable quantities of ethanol are required for this procedure.

b Formaldehyde is more widely available and is effective in concentrations of 3 - 10% of the commercial formulation However, it shrinks and hardens specimens, collector, and laboratory analyst without favor! In order to minimize bad effects from formalin, neutralized formalin is recommended Mollusc shells will eventually disintegrate in acid formalin

c Properly preserved benthos samples may be retained indefinitely, thereby enhancing their utility.

d Refrigeration or icing is very helpful.

V MICROFAUNA AND PERIPHYTON (OR AUFWUCHS) SAMPLING

A This is a relatively new area which promises to be of great importance The microfauna of mud and sand bottoms may be studied to some extent from collections made with the various devices mentioned above In most cases however, there is considerable loss of the smaller forms

B Most special microfauna samplers for soft bottoms are essentially modified core samplers in which an effort is made to bring up an undisturbed portion of the bottom along with the immediately overlying water. The best type currently seems to be the Enequist sampler which weighs some 35 kg. and takes a 100 sq cm sample 50 cm deep.

C Microfauna from the surface of hard sand or gravel bottoms may be sampled by the Hunt vacuum sampler. This has a bell-shaped "sampling" tube sealed by glass diaphragm On contact with the bottom, the glass is automatically broken and the nearly bottom material is swept up into a trap.

D Periphyton attached to or associated with hard surfaces such as rock or wood may be sampled by scraping or otherwise removing all surface material from a measured area. The periphyton, however, is more effectively quantitatively sampled by artificial substrate techniques described above.

VI THE COLLECTION, OR SAMPLING OF PLANKTON PLATE IV

A Phytoplankton A Planned Program is Desirable

1 A planned program of plankton analysis should involve periodic sampling at weekly or even more frequent intervals.

2 A well-planned study or analysis of the growth pattern of plankton in one year will provide a basis for predicting conditions the following year since seasonal growth patterns tend to repeat themselves from year to year.

a Since the seasons and the years differ, records accumulated over the years become more useful.

b As the time for an anticipated bloom of some troublesome species approaches, the frequency of analyses may be increased.

3 Detection of a bloom in its early stages will facilitate more economical control

B Field Aspects of the Analysis Program

1 Two general aspects of plankton analysis are commonly recognized· quantitative and qualitative.

a Qualitative examination tells what is present.

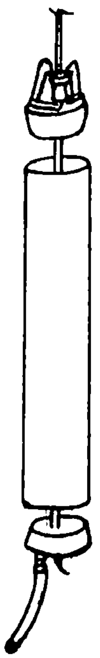
b Quantitative tells how much.

c Either approach is useful, a combination is best.

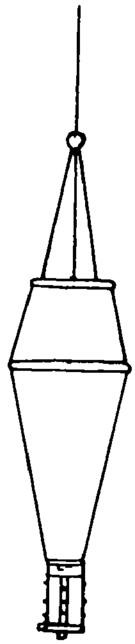
2 Equipment for collecting samples in the field is varied.

a A half-liter bottle will serve for surface samples of phytoplankton, if carefully taken.

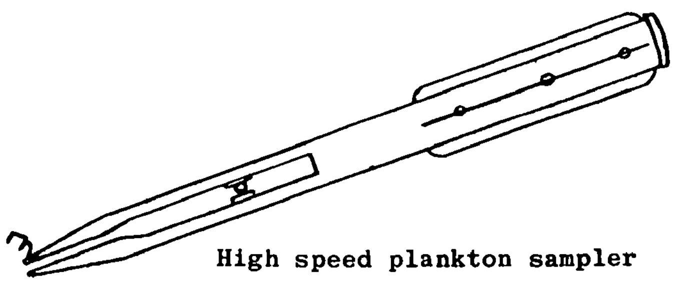
PLANKTON SAMPLERS



Kemmerer



Wisconsin net



High speed plankton sampler

PLATE IV

b A Kemmerer, Nansen, or other special sampler (small battery operated pumps are time saving) is suggested for depth samples.

c Plankton nets concentrate the sample in the act of collecting and also capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however. For phytoplankton, #20 or #25 size nets are commonly used. Usually a net diameter of 5 - 10 inches is sufficient. The smaller forms however, are lost through any net.

C Zooplankton Collecting

1 Since zooplankton have the ability to swim away from water bottles, etc. nets towed at moderately fast speed are used for their capture. Number 12 nets (aperature size 0.119 mm, 125 meshes 1 inch) or smaller numbered net sizes are commonly used. A net diameter greater than 5" is preferred.

Frequently half meter nets or larger are employed. These may be equipped with flow measuring devices for measuring the amount of water entering the net.

2 Other instruments such as the Clark-Bumpus, Gulf-Stream, Hardy continuous plankton recorder, and high-speed instruments are used for collecting zooplankton, also.

3 The devices used for collecting plankton capture both the plant and animal types. The mesh size (net no.) is a method for selecting which category of plankton is to be collected.

D The Location of Sampling Points

1 Both shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot. The surface film is often significant.

b "Deep" samples should be taken at such intervals between surface and bottom as circumstances dictate. In general, the entire water column should be sampled as completely as practicable, and the plankton from each level recorded separately.

2 For estuarine plankton, it is necessary to sample different periods in the stage of the tide, otherwise samples would be biased to a given time, or type of water carried by the tidal currents.

3 Plankton is subjected to the force of the winds and currents. As a result, the plankton is often in patches or "wind rows". For this reason when using a net, it is often desirable to tow the net at right angles to the wind or current.

4 Not only are all plankton likely to be horizontally discontinuous, but zooplankton especially tend to be numerous near the bottom in daylight, but distributed more evenly through the water column at night. Therefore, a series of tows or samples at different depths is necessary to obtain a complete sampling. One technique often employed is to take an oblique tow from the bottom to the top of the water column.

5 Pilot studies to indicate sampling locations and intervals are often mandatory. Some studies require random sampling points.

6 The number of sampling stations that should be established is limited by the capability of the laboratory to analyze the samples, but should approach the needs of the objectives as closely as possible.

7 Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection as in II above.

8 Provisions should be made for the field stabilization of the sample until the laboratory examination can be made if more than an hour or so is to elapse.

a Refrigeration or icing is very helpful, but ice should never be placed in the sample.

b Preservation by 5% formalin is widely used but badly shrinks animals and makes all forms brittle.

c Lugols solution is a good preservative.

d Ultra-violet sterilization is sometimes used in the laboratory to retard the decomposition of plankton.

e A highly satisfactory merthiolate preservative has been developed by the FWPCA and described by Weber (1968).

VII COLLECTING FISH AND OTHER NEKTON PLATES V, VI

A Fish and other nekton must be sought in the obscure and unlikely areas as well as the obvious locations in order for the collection to be complete. Several techniques should be employed wherever possible (this is appropriate for all biota). It is advisable to check with local authorities to inform them of the reasons for sampling, because many of the techniques are not legal for the layman. In this area, perhaps more than any other, professionally trained workers are important. Also, there must be at least one helper, as a single individual always has difficulty in pulling both ends of a 20 foot seine simultaneously! The more common techniques are listed below.

B Seines

1 Straight seines range from 4-6 feet and upwards in length. "Common sense" minnow seines with approximately 1/4 inch mesh are widely used along shore for collecting the smaller fishes.

2 Bag seines have an extra trap or bag tied in the middle which helps trap and hold fish when seining in difficult situations.

C Gill nets are of use in offshore and/or deep waters. They range in length from approximately 30 yards upward. A mesh size is designed to catch a specified size of fish. The trammel net is a variation of the gill net.

D Traps range from small wire boxes or cylinders with inverted cone entrances to semi-permanent weirs a half mile or more in length. All tend to induce fish to swim into an inner chamber protected by an inverted cone or V - shaped notch to prevent escape. Current operated rotating fish traps are also very effective (and equally illegal) in suitable situations.

E Trawls are submarine nets, usually of considerable size, towed by vessels at speeds sufficient to overtake and scoop in fish, etc. The mouth of the net must be held open by some device such as a long beam (beam trawl) or two or more vanes or "otter boards" (otter trawl). Plate III

1 Beam and otter trawls are usually fished on the bottom, but otter trawls when suitably rigged are now being used to fish mid-depths.

2 The midwater trawl resembles a huge plankton net many feet in diameter. It is proving very effective for collecting at mid-depths.

FISH NETS

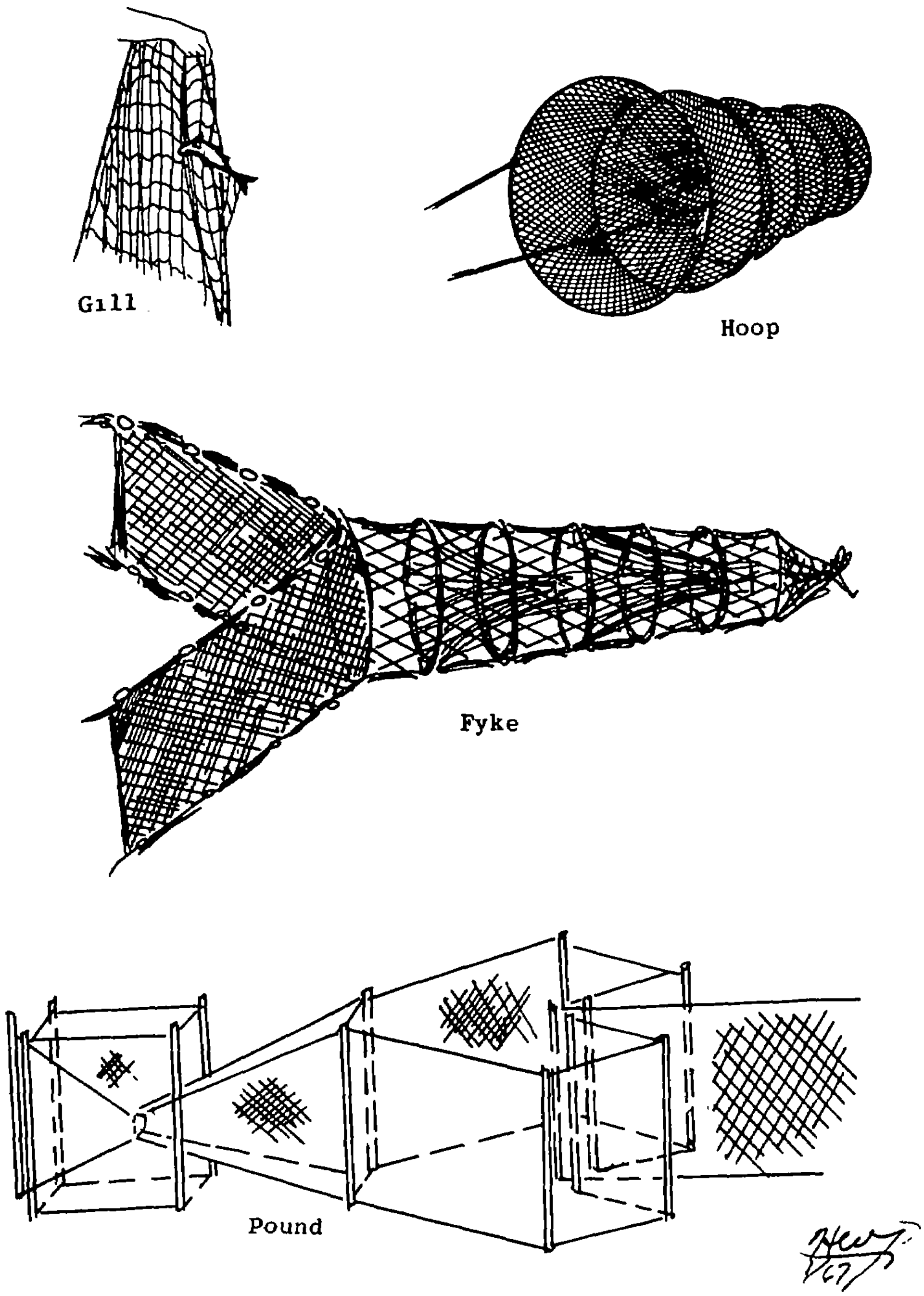


PLATE V

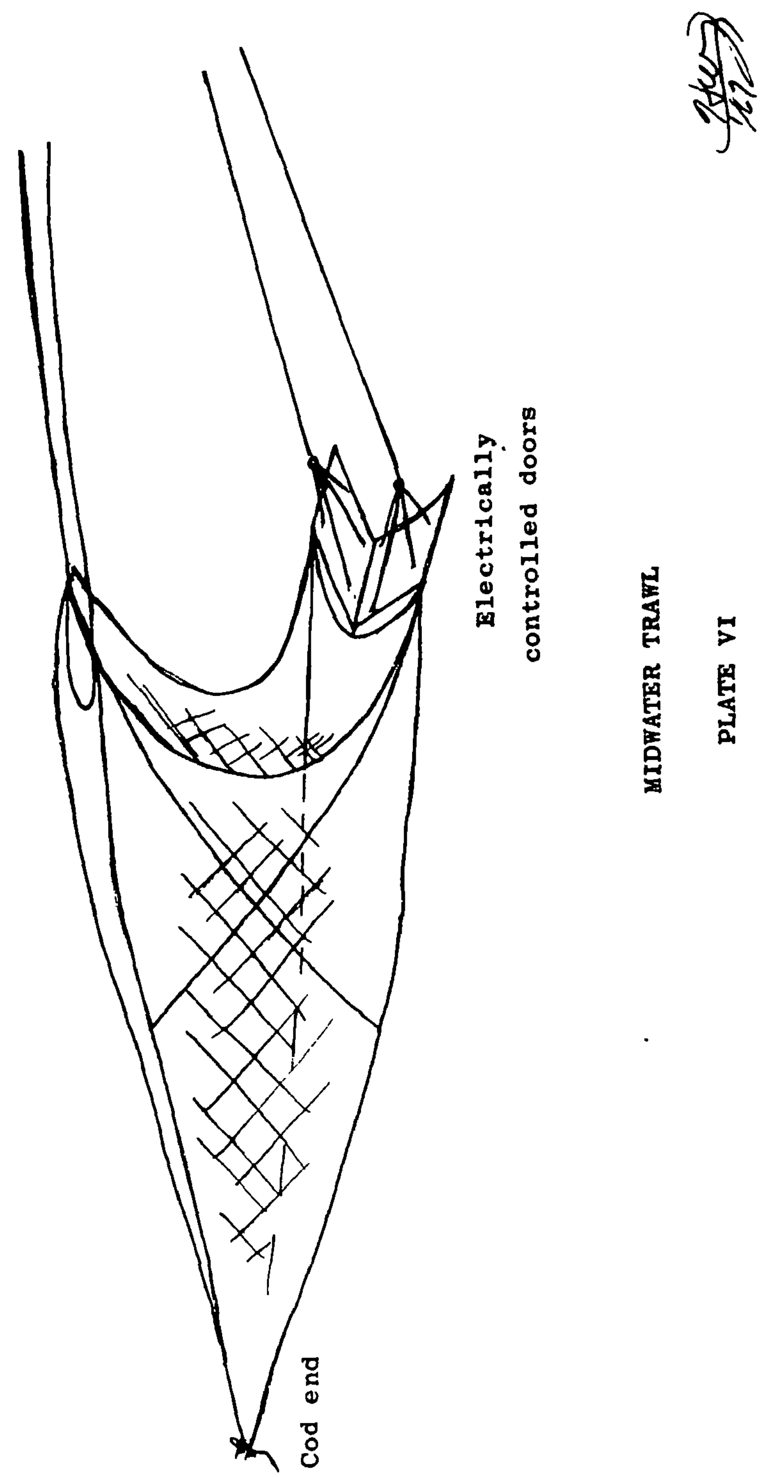

Electrically controlled doors
MIDWATER TRAWL
PLATE VI
Cod end

PLATE VI

Numerous special designs have been developed. Plate VI

F Electric seines and screens are widely employed by fishery workers in small and difficult streams. They may also be used in shallow water like areas with certain reservations.

G Poisoning is much used in fishery studies and management. Most widely used and generally satisfactory is rotenone in varying formulations, although many others have been employed from time to time, and some appear to be very good. Under suitable circumstances, fish may even be killed selectively according to species.

H Personal observation by competent personnel, and also informal inquiries and discussions with local residents will often yield information of real use. Many laymen are keen observers, although they do not always understand what they are seeing. The organized creel census technique yields data on what and how many fish are being caught.

I Angling remains in its own right a very good technique in the hands of the skilled practitioner, for determining what fish are present. Spear-fishing also is now being used in some studies.

J Fish and other nekton are often tagged to trace their movements during migration and at other times. Miniature radio transmitters can now be attached or fed to fish (and other organisms) which enable them to be tracked over considerable distances. Physiological information is often obtained in this way. This is known as telemetry.

VIII SPECIAL REQUIREMENTS ON BOATS

Handling biological collections (as contrasted to chemical and physical sampling) on board boats differs with the size of the craft and the magnitude of operations. Some possible items are listed below. Hoisting and many other types of gear are used in common with other types of collection, and will not be listed.

A Special Laboratory Room(s)

B Constant flow of Clean water for culturing organisms. (Selection of materials and design of a system to insure non-toxic water may be very troublesome but very important.)

C Live Box built into ship at water level

D Refrigeration System(s)

1 For controlling temperature of experimental organisms in laboratory.

2 For deep-freezing and storage of specimens to be examined later.

E Storage Space (Unrefrigerated)

F Facilities for the safe storage and use of microscopes and other laboratory equipment.

G Facilities for the safe storage and use of deck equipment.

H Administrative access to the Captain and Technical Leader in order to coordinate requirements for biological collection (such as a slow plankton tow) with those for other collections.

I Safety of personnel working in and around boats, as well as in other field activities should be seriously considered and promoted at all times.

IX OTHER TYPES OF BIOLOGICAL FIELD STUDIES INCLUDE

A Productivity Studies of Many Types

B Life Cycle and Management

C Distribution of Sport or (potentially) Commercial Species

D Scattering Layers and Other Submarine Sound Studies

E Artificial Culture of Marine Food Crops

F Radioactive Uptake

G Growth of Surface-Fouling Organisms

H Marine Borers

I Dangerous Marine Organisms

J Red Tides

K Others

X SOURCES OF COLLECTING EQUIPMENT

Many specialized items of biological collecting equipment are not available from the usual laboratory supply houses. Consequently, the American Society of Limnology and Oceanography has compiled a list of companies handling such items and released it as "Special Publication No. 1, Sources of Limnological and Oceanographic Apparatus and Supplies." Available from the Secretary of the Society.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

I Invertebrates which are part of the benthos, but under certain conditions become carried downstream in appreciable numbers, are known as drift.

A Groups which have members forming a conspicuous part of the drift include the insect orders Ephemeroptera, Trichoptera, Plecoptera and the crustacean order Amphipoda.

B Other invertebrate groups exhibit drift patterns.

II THREE BASIC TYPES OF DRIFT ARE RECOGNIZED

A Catastrophic Drift

Floods wash numerous benthic organisms downstream. Application of pesticides may also cause such drift.

B Constant Drift (Incidental or Adventitious)

Organisms are constantly being dislodged from the substrate during normal activities and carried downstream.

C Periodic (Diel) or Behavioral Drift

In contrast to the other categories, this is a specific behavior pattern and related to circadian activity rhythms.

1 Seasonal drift occurs, for example, in some maturing stoneflies which drift downstream for emergence. This is another reason for a serious consideration of drift in bottom fauna sampling since such presence of stoneflies could easily be misinterpreted.

2 Periodic or diel drift occurs in peaks for successive 24-hour periods.

a Night-active. Light intensity is the phase-setting mechanism.

b Day-active. Water temperature appears to be the phase-setter.

III DIEL DRIFT

A Diel activity rhythms generally include two peaks during the 24-hour period, one major and the other minor.

1 The bigeminus type in which the major peak occurs first (after sunset).

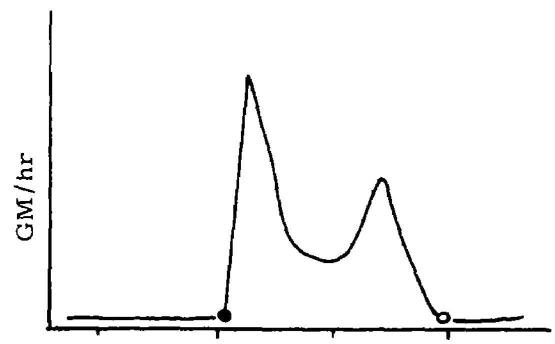

FIGURE 1

2 The alternans pattern with the major peak occurring last.

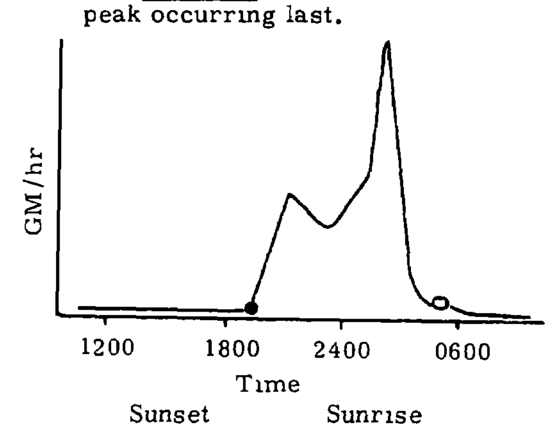

FIGURE 2

B Drift Rate and Density (Waters, 1969)

1 Drift rate defined is "...the quantity of organisms passing a width transect or portion thereof, per unit time,

it is a measure of displacement or the movement of organisms from one place to another."

2 Drift density "...is the quantity of organisms per unit volume of water, in much the same way as plankton density can be defined."

IV IMPLICATIONS FOR BIOLOGICAL SAMPLING

A The drift from productive upstream reaches may support a fish population existing in relatively barren stream sections.

B Drift will colonize artificial substrates, such as suspended rock baskets, when placed in such habitats.

C A bottom sampler such as the Surber, could also be sampling drift when only resident benthic organisms are intended to be collected. This would depend on the hour of collection and length of time the Surber sampler is in the water.

D Application of drift studies have been widely used in pesticide related studies. In conjunction with such studies, Dimond concluded that·

1 The status of drift is a much better indicator of the steady state and of total productivity than is the status of the bottom fauna.

2 Bottom sampling, however, is superior when analyzing survival and recovery of the quality of population.

3 A combination of both in such a sampling program would be most likely to yield the most useful data.

E Drift sampling techniques have been useful for recovery of large numbers of sand-dwelling mayflies, which were once rarely collected.

V MAJOR TAXA INVOLVED IN DRIFT

A The crustacean order Amphipoda

1 Gammarus species

2 Hyalella azteca

B The Insect Orders

1 Ephemeroptera

Baetis species (apparently universal)

2 Plecoptera

3 Trichoptera

4 Diptera
Simuliidae

5 Elmidae

C The main groups exhibiting very high drift rates include· Baetis, some Gammarus species, and some Simuliidae.

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ARTIFICIAL SUBSTRATES

I INTRODUCTION THE NATURE OF ARTIFICIAL SUBSTRATES

A Artificial substrates are anything deliberately placed in the water for the purpose of providing a place for benthic or attached (sessile, sedentary, etc.) organisms to grow on or in. This is in contrast to "bait" which is used as an attractant.

B Their origins for commercial use, or human food production are rooted in antiquity. Some examples are

1 Ropes, poles, brush, concrete structures, and other objects thrust into the bottom, or suspended in estuarine waters to catch and grow oysters and mussels (cultural techniques), known virtually around the world.

2 Straw or reed tepees planted in shallow alkaline lakes (in Mexico for example) to catch the eggs of Corixids (Insecta. Order Hemiptera, back-swimmers). Eggs are harvested by drying and brushing them off onto white sheets. Used for human food.

C The fouling of ships bottoms, piling, etc. by barnacles and other marine life is an "artificial substrate in reverse".

D The use of aggregate to support a zoogloeal mass of micro-biota in a trickling filter, thus simulating a riffle area in a surface stream, is a modern concept to harness and make use of "consumer" and "reducer" elements of a community in order to dissipate the energy (oxidize, exhaust the food value) contained in sewage.

II ECOLOGICAL BASIS

A Artificial substrates are based on the "laws of organismal distribution."

1 Any given kind of organisms tends to be present (inhabit) in all available suitable habitats.

2 Any given habitat tends to be inhabited by all suitably adapted kinds of organisms.

B A "substrate" being an object (or group of objects) constitutes a habitat suitable for sessile or attached organisms, and also those that naturally burrow in, crawl over, or otherwise live associated with objects. Natural objects here could mean the bottom, stones, sticks (floating or sunk), etc.

C Organisms that would not be attracted to substrates would be plankton and nekton (fish and larger swimming invertebrates).

D Ecological Succession

Colonization is rapid in a biologically productive water, and normally reaches a stable climax* community in about a month. A typical outline of successive forms to appear in a freshwater situation, for example, might be as follows.

1 Periphyton (slime forming) stage (see also below)

a Bacteria - within an hour

b Diatoms - within the first day

c Other micro-algae - within the first day

d Protozoa - within the first day

2 Macroinvertebrate dominated stage (see also below)

a Primary attached or sedentary colonizers - second to third day

1) Net caddisflies

2) Bryozoa

3) Cordylophora caspia

4) Hydra

*A community which has achieved a point of no further change, under a given set of environmental conditions. Time scale may vary with circumstances.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

b Primary foragers

1) Mayflies

2) Stoneflies

3) Midges

c Secondary attached or sedentary colonizers.

1) Sponges

2) Filamentous algae

d Adventitious forms

1) Crustaceans

2) Flatworms

3) Leeches

4) Snails

5) Other

3 Artificial substrates in a marine environment proceed through similar stages, except that the macroinvertebrate stage may be more subject to variation in the attachment of broods of barnacle, oyster, and other larvae resulting from greater numbers of types present, tidal current variation, meteorological conditions, etc.

III ARTIFICIAL SUBSTRATES AS SCIENTIFIC COLLECTING DEVICES

A A review of the history of artificial substrates for collecting microorganisms (aufwuchs) (Cooke, 1956) indicates that glass microscope slides were first used for this purpose about 1915. Wood or metal panels appear, however, to have been deliberately exposed for the scientific collection of larger organisms at least since approximately the turn of the century, and probably long before that (Visscher, 1928).

B Biological Applications

The principles of the artificial substrate remain the same, regardless of the community sampled. Two general types of communities and associated samplers have been employed

1 Periphyton (or aufwuchs) samplers

Periphyton is the community of slime forming microorganisms which is the first to attach to objects newly exposed under water. This community is generally considered to provide an anchor layer to which other higher forms of life can more readily attach. It tends to persist until overgrown or displaced by larger organisms, and then in turn can be found spreading over the surfaces of these same larger plants and animals.

2 Periphyton has been widely studied as it appears on 1 X 3 glass microscope slides which are equally convenient to expose in the field and to study in the laboratory.

3 Particular studies have included

a The original bacterial and fungal slime

b Diatom identification and counts

c Identification and counts of other microscopic algae

d Protozoans

e Primary productivity

4 The macroinvertebrate community is sampled by a great variety of devices such as those cited below. The organisms are usually removed from the substrate for study. Applications have included the following:

a General study of the macroinvertebrate community b Estimates of productivity

c Studies of the life cycle of particular species

d Studies of the influence of the substrate on the attachment of sessile forms

1) The influence of toxic paints for the prevention of fouling organisms

2) Wood panels to study the penetration of boring molluscs and crustaceans

C Effect of type of device on what is collected

1 Wood boring organisms like teredo worms (Mollusca, Pelecypoda) or gribbles (Arthropoda, Isopoda) would obviously be attracted primarily to wood (although some are known to bore in other materials).

2 Delicate forms and crawling forms would be most likely to be collected on devices having a shape to protect against strong currents

3 Those with strong attachments could endure swift currents, often, surprisingly, even during periods of original attachment (ex. byssus attached clams which are also benthic forms).

4 Bottom burrowers would be most likely collected in artificially contained portions of bottom material.

D Effect of Location

1 The depth at which a sampler is suspended may influence the organisms attracted.

2 Location in or out of a current, direct sunlight, etc , will influence the take.

E Some Types of Devices

1 Cement plates, panels, and blocks

2 Ceramic tiles

3 Wood blocks

4 Metal plates

5 Glass slides - 1 X 3 inch micro slides are used by many workers. Numerous devices are employed to hold them. They are generally either floated (Weber and Raschke 1966) or suspended in racks, anchored to submerged bricks or other objects.

6 Plastic petri dishes

Burbanck and Spoon utilized an ordinary 50 X 12 mm plastic petri dish for collecting sessile protozoa. Sickle modified this by using a styrofoam cup (6 oz. size) with the bottom third being cut off. The lower unit of the plastic dish is easily wedged into place in the cup and the device is simply held by a nylon line on a rope held in place by an appropriate anchor and float.

The cup which tends to float is so held that the petri dish bottom is in a horizontal position and bottom side up.

7 Multiple plate (Hester and Dendy, 1962)

a Common current procedure utilizes 3-inch squares of 1/4 inch thick Masonite separated by 1-inch square spacers.

These may be

b Threaded on an eye bolt or long rod.

c Suspended by a loop of nylon cord.

8 Baskets or trays of bottom-type material

a Trays of bottom material sunk in the surface layer of the bottom.

b Baskets of stones suspended in the water (Anderson and Mason, 1966).

9 Boxes, cages, bundles, etc., of brush, reeds, or artificial material.

10 Polyethylene tapes

11 Plastic webbing

Minnesota Mining and Manufacturing Company conservation web no. 200.

12 Styrofoam

13 Glass coverslips

Small slips are floated on the surface of the water. Highly useful for protozoa and rotifers. Remove and place on a micro slide. Examine as a wet mount.

F Retrieval is an acute problem with all of these samplers.

1 Physical factors

a Relocation

b Floods and drift

c High water

2 Well marked samplers or floats are naturally vulnerable to the public, resulting in disturbed, damaged, or destroyed sample gear.

a This has been overcome by an ingenious submerged float and recovery line device. The weak link in a submerged recovery line is a modified flash bulb. An electronic device actuated by an underwater gun breaks the bulb allowing the float and attached line to surface. (Ziebell, McConnell, and Baldwin)

b This unit has been further modified by Fox (University of Georgia Cooperative Fishery Unit) who used an inexpensive detonator, "Seal Salute". The latter is an inexpensive fused charge designed for underwater explosion.

IV ARTIFICIAL SUBSTRATES OR SAMPLERS, AND WATER QUALITY

A Artificial substrates provide a habitat ("place to live"). It follows from the laws of distribution (II A 1 and 2 above), that the community which inhabits a device will be governed by the physical nature or structure interacting with the characteristics of the surrounding water (velocity, temperature, chemical characteristics, etc.). Since the nature of the sampler is controlled, it is evident that the characteristics of the water constitute the variable factor.

B Water Quality Surveillance

1 Similar substrates suspended side by side in the same water tend to accumulate (essentially) the same communities and quantities of organisms.

2 Similar substrates suspended in different waters accumulate different communities and quantities.

3 Ergo: different communities and quantities collected from similar substrates at different places and times, probably indicate different water qualities.

a These may be natural (seasonal, diurnal, etc.)

b Or they may be a result of human influences (pollution)

c A series of samplers the length of a stream, lake, or estuary can suggest "steady state" differences in water quality.

d A series of samplers exposed over a period of time at a given site can suggest changes of water quality in time.

4 The artificial substrate thus essentially constitutes an in-situ bioassay of the water.

C Interpretation and Significance of Collections

1 The unit of comparison is most appropriately taken as "the sampler". The artificial substrate by definition is not the natural local bottom material, and unless it consists of a portion of that bottom which has been actually removed and replaced in an artificial container (III-D-7) the composition and magnitude of the community it contains may or may not bear a definitive relationship to the actual natural problem The take of the artificial substrate thus may have relatively little relationship to the take of a Petersen or an Ekman grab (dredge).

2 Comparisons between different types of samplers are likewise hazardous Each is what it is, and if they are different they are not identical, thus the biota each collects cannot be expected to be identical (CF II A)

3 Artificial substrates should generally be compared on a "sampler vs sampler" basis, or for periphyton, "unit area vs unit area".

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ATTACHED GROWTHS
(Periphyton or Aufwuchs)

I The community of attached microscopic plants and animals is frequently investigated during water quality studies. The attached growth community (periphyton) and suspended growth community (plankton) are the principal primary producers in waterways--they convert nutrients to organic living materials and store light originating energy through the processes of photosynthesis. In extensive deep waters, plankton is probably the predominant primary producer. In shallow lakes, ponds, and rivers, periphyton is the predominant primary producer. During the past two decades, investigators of microscopic organisms have increasingly placed emphasis on periphytic growths because of inherent advantages over the plankton when interpreting data from surveys on flowing waters

A Blum (1956) "....workers are generally agreed that no distinctive association of phytoplankton is found in streams, although there is some evidence of this for individual zooplankters (animals) and for a few individual algae and bacteria. Plankton organisms are often introduced into the current from impoundments, backwater areas or stagnant arms of the stream.... Rivers whose plankton is not dominated by species from upstream lakes or ponds are likely to exhibit a majority of forms which have been derived from the stream bottom directly and which are thus merely facultative or opportunistic plankters. "

B "The transitory nature of stream plankton makes it nearly impossible to ascertain at which point upstream agents producing changes in the algal population were introduced, and whether the changes occurred at the sampling site or at some unknown point upstream. In contrast, bottom algae (periphyton) are true components of the stream biota. Their sessile-attached mode of life subjects them to the quality of water continuously flowing over them. By observing the longitudinal distribution of bottom algae within a stream, the sources of the agents producing the change can be traced (back-tracked)" (Keup, 1966).

II TERMINOLOGY

A Two terms are equally valid and commonly in use to describe the attached community of organisms. Periphyton literally means "around plants" such as the growths overgrowing pond-weeds, through usage this term means the attached film of growths that rely on substrates as a "place-to-grow" within a waterway. The components of this growth assemblage consists of plants, animals, bacteria, etc. Aufwuchs is an equally acceptable term [probably originally proposed by Seligo (1905)]. Aufwuchs is a German noun without equivalent english translation, it is essentially a collective term equivalent to the above American (Latin root) term - Periphyton. (For convenience, only, PERIPHYTON, with its liberal modern meaning will be used in this outline.)

B Other terms, some rarely encountered in the literature, that are essentially synonymous with periphyton or describe important and dominant components of the periphytic community are Nereiden, Bewuchs, Laison, Belag, Besatz, attached, sessile, sessile-attached, sedentary, seeded-on, attached materials, slimes, slime-growths, and coatings.

The academic community occasionally employs terminology based on the nature of the substrates the periphyton grows on (Table 1).

TABLE 1

Periphyton Terminology Based on Substrate Occupied

Substrate	Adjective
various	epiholitic, nereiditic, sessile
plants	epiphytic
animals	epizooic
wood	epidendritic, epixylonic
rock	epilithic

[After Srameck-Husek (1946) and via Sladeckova (1962)] Most above listed latin-root adjectives are derivatives of nouns such as epihola, epiphyton, spizoa, etc.

III Periphyton, as with all other components of the environment, can be sampled qualitatively (what is present) and quantitatively (how much or many are present).

A Qualitative sampling can be performed by many methods and may extend from direct examination of the growths attached to a substrate to unique "cuttings" or scrapings. It may also be a portion of quantitative sampling.

B Quantitative sampling is difficult because it is nearly impossible to remove the entire community from a standardized or unit area of substrate.

1 Areas scraped cannot be determined precisely enough when the areas are amorphous plants, rocks or logs that serve as the principal periphyton substrates.

2 Collection of the entire community within a standard area usually destroys individual specimens thereby making identification difficult (careful scraping can provide sufficient intact individuals of the species present to make qualitative determinations), or the process of collection adds sufficient foreign materials (i.e. detritus, substrate, etc.) so that some commonly employed quantitative procedures are not applicable.

IV Artificial substrates are a technique designed to overcome the problems of direct sampling. They serve their purpose, but cannot be used without discretion. They are objects standardized as to surface area, texture, position, etc. that are placed in the waterway for pre-selected time periods during which periphytic growths accumulate. They are usually made of inert materials, glass being most common with plastics second in frequency. Over fifty various devices and methods of support or suspension of the substrates have been devised (Sladeckova, 1962) (Weber, 1966) (Thomas, 1968).

V ARTIFICIAL SUBSTRATE PLACEMENT

A Position or Orientation

1 Horizontal - Includes effects of settled materials.

2 Vertical - Eliminates many effects of settled materials.

B Depth (light) - A substrate placed in lighted waters may not reflect conditions in a waterway if much of the natural substrate (bottom) does not receive light or receives light at reduced intensity. (Both excessive light and a shortage of light can inhibit growths and influence the kinds of organisms present.)

C Current is Important

1 It can prevent the settling of smothering materials.

2 It flushes metabolic wastes away and introduces nutrients to the colony.

VI THE LENGTH OF TIME THE SUBSTRATE IS EXPOSED IS IMPORTANT.

A The growths need time to colonize and develop on the recently introduced substrate.

B Established growths may intermittently break-away from the substrate because of current or weight induced stresses, or "over-growth" may "choke" the attachment layers (nutrient, light, etc. restrictions) which then weaken or die allowing release of the mass.

C A minimum of about ten days is required to produce sufficient growths on an artificial substrate, exposures exceeding a longer time than 4-6 weeks may produce "erratic results" because of sloughing or the accumulation of senile growths in situations where the substrate is artificially protected from predation and other environmental stresses.

VII Determining the variety of growths present is presently only practical with microscopic examination. (A few micro-chemical procedures for differentiation show promise-- but, are only in the early stages of development.)

VIII DETERMINING THE QUANTITY OF GROWTH(S)

A Direct enumeration of the growths while attached to the substrate can be used, but is restricted to the larger organisms because (1) the problem of maintaining material in an acceptable condition under the short working distances of the objective lenses on compound microscopes, and (2) transmitted light is not adequate because of either opaque substrates and/or the density of the colonial growths.

B Most frequently, periphyton is scraped from the substrate and then processed according to several available procedures, the selection being based on the need, and use of the data.

1 Aliquots of the sample may be counted using methods frequently employed in plankton analysis.

a Number of organisms

b Standardized units

c Volumetric units

d Others

2 Gravimetric

a Total dry weight of scrapings

b Ash-free dry weight (eliminates inorganic sediment)

c A comparison of total and ash-free dry weights

3 Volumetric, involving centrifugation of the scrapings to determine a packed biomass volume.

4 Nutrient analyses serve as indices of the biomass by measuring the quantity of nutrient incorporated.

a Carbon

1) Total organic carbon

2) Carbon equivalents (COD)

b Organic nitrogen

c Phosphorus - Has limitations because cells can store excess above immediate needs.

d Other

5 Chlorophyll and other bio-pigment extractions.

6 Carbon-14 uptake

7 Oxygen production, or respiratory oxygen demand

IX EXPRESSION OF RESULTS

A Qualitative

1 Forms found

2 Ratios of number per group found

3 Frequency distribution of varieties found

4 Autotrophic index (Weber)

5 Pigment diversity index (Odum)

B Quantitative

1 Areal basis--quantity per square inch, foot, centimeter, or meter. For example

a 16 mgs/sq. inch

b 16,000 cells/sq. inch

2 Rate basis. For example

a 2 mgs/day, of biomass accumulation

b 1 mg O_2/mg of growth/hour

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APPLICATION OF BIOLOGICAL DATA

I ECOLOGICAL DATA HAS TRADITIONALLY BEEN DIVIDED INTO TWO GENERAL CLASSES:

A Qualitative - dealing with the taxonomic composition of communities

B Quantitative - dealing with the population density or rates of processes occurring in the communities

Each kind of data has been useful in its own way.

II QUALITATIVE DATA

A Certain species have been identified as

1 Clean water (sensitive) or oligotrophic

2 Facultative, or tolerant

3 Preferring polluted regions (see Fjerdinstad 1964, 1965, Gaufin & Tarzwell 1956, Palmer 1963, 1969, Rawson 1956, Teiling 1955)

B Using our knowledge about ecological requirements the biologist may compare the species present

1 At different stations in the same river (Gaufin 1958) or lake (Holland 1968)

2 In different rivers or lakes (Robertson and Powers 1967)

or changes in the species in a river or/lake over a period of several years. (Carr & Hiltunen 1965, Edmondson & Anderson 1956, Fruh, Stewart, Lee & Rohlich 1966, Hasler 1947).

C Until comparatively recent times taxonomic data were not subject to statistical treatment

III QUANTITATIVE DATA: Typical Parameters of this type include

A Counts - algae/ml, benthos/m^2, fish/net/day

B Volume - mm^3 algae/liter

C Weight - dry wgt, ash-free wgt

D Chemical content - chlorophyll, carbohydrate, ATP, DNA, etc

E Calories (or caloric equivalents)

F Processes - productivity, respiration

IV Historically, the chief use of statistics in treating biological data has been in the collection and analysis of samples for these parameters. Recently, many methods have been devised to convert taxonomic data into numerical form to permit:

A Better communication between the biologists and other scientific disciplines

B Statistical treatment of taxonomic data

C In the field of pollution biology these methods include:

1 Numerical ratings of organisms on the basis of their pollution tolerance

(saprobic valency: Zelinka & Sladecek 1964)

(pollution index: Palmer 1969)

2 Use of quotients or ratios of species in different taxonomic groups (Nygaard 1949)

3 Simple indices of community diversity

a Organisms are placed in taxonomic groups which behave similarly under the same ecological conditions. The number of species in these groups found at "healthy" stations is compared to that found at "experimental" stations. (Patrick 1950)

b A truncated log normal curve is plotted on the basis of the number of individuals per diatom species (Patrick, Hohn, & Wallace 1954)

c Sequential comparison index. (Cairns, Albough, Busey & Chanay 1968). In this technique, similar organisms encountered sequentially are grouped into "runs".

$$SCI = \frac{\text{runs}}{\text{total organisms examined}}$$

d Ratio of carotenoids to chlorophyll in phytoplankton populations·

OD_{430}/OD_{665} (Margalef 1968)

OD_{435}/OD_{670} (Tanaka, et al 1961)

e The number of diatom species present at a station is considered indicative of water quality or pollution level. (Williams 1964)

f $\frac{\text{number of species (S)}}{\text{number of individuals (N)}}$

g $\frac{\text{number of species (S)}}{\text{square root of number of individuals} (\sqrt{N})}$

h $\frac{S - 1}{\log_e N}$ (Menhinick 1964)

i $d = \frac{\Sigma n_i (n_i - 1)}{N (N - 1)}$ (Simpson 1949)

where n_i = number of individuals belonging to the i-th species, and

N = total number of individuals

j Information theory

The basic equation used for information theory applications was developed by Margalef (1957).

$$I = \frac{1}{N} \log_2 \frac{N!}{N_a! N_b! \ldots N_s!}$$

where I - information/individual, $N_a, N_b \ldots N_s$ are the number of individuals in species a, b, ... s, and N is their sum.

This equation has also been used with

1) The fatty acid content of algae (McIntire, Tinsley, and Lowry 1969)

2) Algal productivity (Dickman 1968)

3) Benthic biomass (Wilhm 1968)

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PROCEDURES FOR FISH KILL INVESTIGATIONS

I INTRODUCTION

Fish kills in natural waters, though unfortunate, can in many instances indicate poor water quality leading to investigations which may improve water quality. Prompt investigations should be organized and conducted so that the resultant data implicates the correct cause. Fish kills tend to be highly controversial, usually involving the general public as well as a number of agencies Therefore, the investigator can expect his findings to be disputed, quite possibly in a court of law

The following procedures are presented as a working guide for investigating and reporting fish kills as developed by the personnel of The Lower Mississippi River Comprehensive Project (FWPCA).

II TYPES AND EXTENT OF FISH KILLS

A Natural Mortalities - Those which are caused through natural phenomena such as, acute temperature change, storms, ice and snow cover, decomposition of natural materials, salinity change, spawning mortalities, parasites, and bacterial or viral epidemics.

B Man caused fish kills - Produced by environmental changes through man's activity, and may be attributed to municipal wastes, industrial wastes, agricultural activities and water control activities.

C One dead fish in a stream may be called a fish kill, however, in a practical sense some minimal range in number of dead fish observed plus additional qualifications should be used in reporting and classifying fish kill investigations. The following definitions should be used as guidelines in reporting fish kill investigations. These qualifications are based on a stream approximating 200 feet in width and 6 feet in depth. For other size streams, adjustments should be made.

1 Minor fish kill considered here as NO fish kill and reported so· 1 - 100 dead or dying fish confined to a small area or stream stretch. Providing this is not a reoccurring or periodic situation. For example, near a waste outfall in which stream dilution plays its part and nullifies the effect of the deleterious material. If this is a reoccurring situation, it could be of major significance and, therefore, investigated.

2 Moderate fish kill· 100 - 1000 dead or dying fish observed. In a stream where dilution has had the chance to play its role involving a mile or so of stream , a number of species are affected, and apparently normal fish can be collected immediately downstream from the observed kill area.

3 Heavy fish kill 10, 000 fish or more observed dead or dying. In a stream where dilution has had the chance to play its part, but ten miles or more of the stream are involved, many species of fish are affected and dying fish may still be observed downstream.

III PREPARATION FOR FIELD INVESTIGATION

A Secure maps of area to be investigated.

1 U.S. Geological Survey maps

a 1/250, 000 scale for general location

b 1/24, 000 for accurately defining the kill area in the field

2 Navigation maps (appropriate agency)

3 Other sources

4 From the data received from the reporting agency, locate the kill area on the map.

a Determine best access points.

b Locate possible known industries, municipalities, or other potential sources of pollution.

c Estimate the possible area to be traveled or inspected on

1) water

2) land

3) both

B Secure sampling equipment and determine size of investigation team needed.

1 Standard equipment to be taken on all investigations (a standard checklist with space for special equipment will often save embarrassment in the field.)

a Thermometer

b Dissolved oxygen sampler

c D.O. bottles

d Winkler D.O. test kit

e Conductivity test meter

f pH test meter or chemical kit

g Sample bottles

h Pencils and note paper

i Current edition of "Standard Methods for the Examination of Water and Wastewater "

2 If preliminary information is available on the possible cause of the kill, consult the latest edition of "Standard Methods" for specific physical and chemical equipment required for collecting, analyzing, or preserving samples possibly containing the suspected causative agent.

3 Form an investigating party

a If only one man is available to make the investigation, preference for choosing the man should be in this order

1) Specialized professional personnel, such as, engineer, chemist, or biologist who is experienced in investigating fish kills and who is capable of adequately reporting the technical aspects of the investigation.

2) A non-specialized professional engineer, chemist, or biologist who has little or no experience in fish kill investigations, but who is capable of adequately reporting the technical aspects of the investigation.

3) A technician who has considerable field experience in pollution and fish kill investigations and who is capable of reporting some of the technical aspects of the investigation.

4) An office technician or other personnel who has had limited field work in pollution investigations.

b If two or more men are needed for the investigation, the party should include at least one person under category (1) above. Preferably, the team should include:

1) A biologist to make a survey of the biological changes.

2) An engineer to make an evaluation of the physical condition of the fish kill area and to make an investigation of an industry or a municipal wastewater treatment plant if needed.

c If a fish kill is observed in its initial state in the field by any one of the people listed under the classification in Section B.3.a., the project office should be informed immediately (after working hours the project director or deputy director should be informed) so that an adequately equipped, specialized investigating party can be formed if needed.

C Contact personnel of the laboratory or laboratories which will participate in analyzing samples. If possible estimate the following and record on sample form No. 1.

1 The number and size of samples to be submitted

2 The probable number and types of analyses required

3 The dates the samples will be received by the laboratory

4 Method of shipment to the laboratory

5 To whom the laboratory results are to be reported

6 The date the results are needed

IV MAKING THE FIELD INVESTIGATION

A Contact the local lay person or official who first observed the kill and reported it.

1 Obtain any additional information which might be helpful which was not reported previously.

2 If possible, retain the reporting party as a guide or invite him to accompany the investigating team.

B Make a reconnaissance of the kill area.

1 Make a decision as to the extent of the kill and if a legitimate kill really has occurred.

2 If a legitimate kill exists take steps to trace or determine the cause.

a Always perform the following physical or chemical tests, during the initial steps of the investigation

1) Temperature

2) pH

3) Dissolved oxygen

4) Specific conductance

While none of these factors may be directly involved in the kill these tests are performed simply and rapidly in the field and can be used as a baseline or starting point for isolating the cause (s) of the kill.

b Record other physical observations such as.

1) Appearance of water, i.e., turbidity, high algal blooms, oily, unusual appearance, etc.

2) Stream flow pattern, i.e., high or low flow, stagnant or rapidly moving water, tide moving in or out, etc. If possible obtain reading from stream gage if one is near kill area.

3) Weather conditions prevailing at the time of the investigation and information on weather immediately prior to the kill

3 Make a rough sketch or define the kill area on a map so that sampling points, sewer outfalls, etc. can be accurately located on a drawing to be included in a final report.

4 Take close-up and distance photographs of:

a Dead fish in the stream in the polluted area.

b The stream above the polluted area.

c Wastewater discharges.

Photographs will often show a marked delineation between the wastewater discharge and the natural flow of water. Pictures taken at a relatively high elevation, (a bridge as opposed to a boat or from a low river bank) will show more and be more effective. Color photographs are also more effective in showing physical conditions of a stream in comparison to black and white prints.

C Sampling Procedures - The extent and method of sampling will depend upon location and upon the suspected cause of the kill.

1 Stream and wastewater sampling.

a Sample the following points when the pollutional discharge is coming from a well defined outfall.

1) The effluent discharge outfall

2) The stream at the closest point above the outfall which is not influenced by the waste discharge

3) The stream immediately below the outfall

4) Other points downstream needed to trace the extent of the pollution

b The sampling should be extensive enough that when all the data is compiled no question will exist as to the source of the pollution which killed the fish.

c The number of samples to be collected at a given cross section will depend principally on the size of the stream.

1) Streams less than 200 feet wide, not in an industrial area usually can be adequately sampled at one point in a section (Figure 1).

2) Streams 200 feet or wider generally should be sampled two or more places in a section immediately above and below the pollutional discharge, where the pollutional waste has adequately mixed with the stream flow one sample may suffice.

3) A number of samples in a cross section may be required on any size of stream to show that the suspected pollutional discharge is coming from a source located in an industrial or municipal complex (Figure 2).

4) Extensive cross sectional sampling on rivers greater than 200 feet wide will be required for kills involving suspected agricultural or other types of mass runoff.

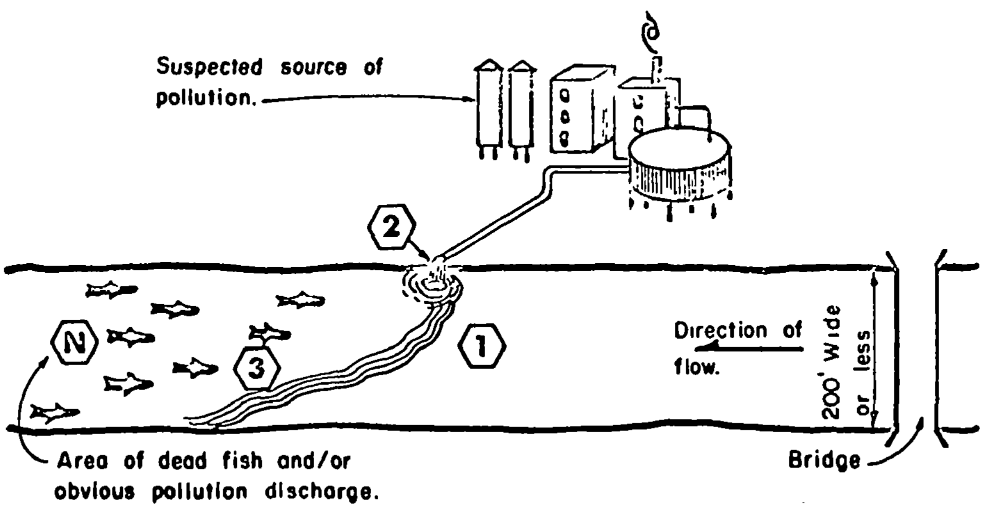

Figure I — Minimum Water Sampling Point On Stream 200 Feet Or Less Wide Involving An Isolated Discharge.

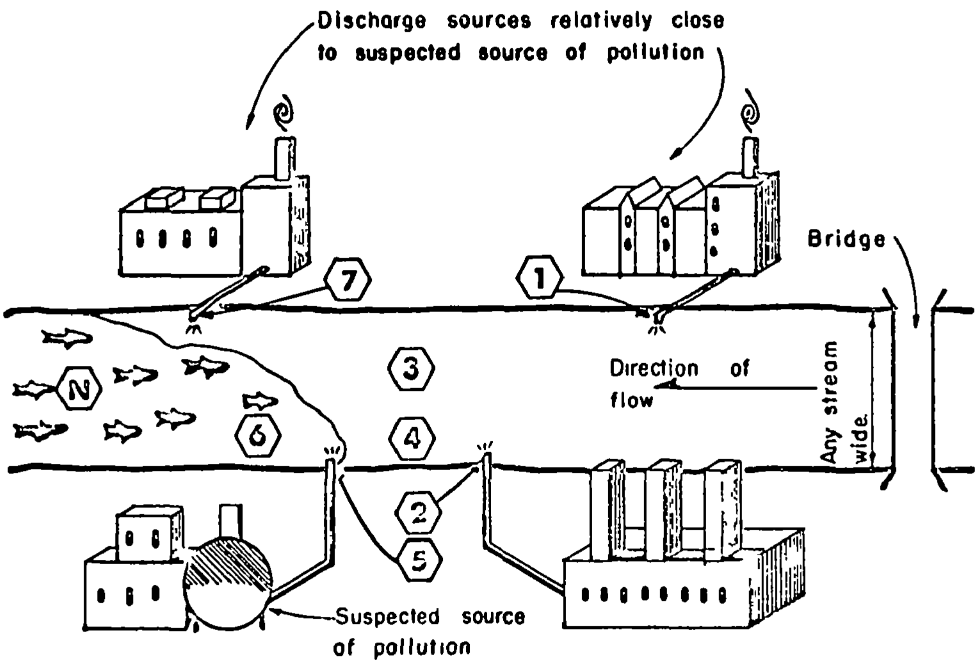

Figure 2 — Minimum Water Sampling Points On A Stream Running Through An Industrial Or Municipal Complex.

PLATE I - RELATIONSHIP OF FISH KILLS TO SOURCE OF TOXICITY

5) Sample depths - On streams 5 feet in depth or less, one mid-depth sample per sampling locations. For streams of greater depths, appropriate sampling judgment should be used, since stratification may be present.

Explanation of Plate I

1) Collection point 1, Figure 1 and points 3 and 4, Figure 2 should be collected as near to the point of pollutional discharge as possible. These points will vary according to stream flow conditions; the pollutional discharges into a slow sluggish stream usually will have a cone of influence upstream of the outfall, whereas, a swift flowing stream usually will not.

2) Collecting an upstream control sample from a bridge within sight of the pollutional discharge would probably be satisfactory in Figure 1 but definitely not in Figure 2.

3) Figures 1 and 2 are given for illustrative purposes only and should be used only as a guide for sampling. Thought must be given to each individual situation to insure adequate, proper sampling. While too many samples are better than too few, effort should be made not to unduly overload the laboratory with samples collected as a result of poor sampling procedures.

2 Biological sampling

a In every investigation of fish or wildlife kills the paramount item should be the immediate collection of the dying or only recently dead organism. This may be done by anyone, sampling and preservation is as follows:

1) Collect 20 plus drops of blood in a solvent rinsed vial, seal same with aluminum foil, cap and freeze.

2) Place bled carcass, or entire carcass if beyond bleeding stage, in plastic bag and freeze. In case no method of freezing is available, icing for a short period prior to freezing may be acceptable. Labeling of both blood and carcass is important.

3) Controls - live specimens of the affected organisms should be obtained from an area within the same body of water which had not been influenced by the causative agent. Once obtained these specimens should be handled in a like manner.

b The number of individuals involved and the species affected should be enumerated in some manner. At most these will be estimates. Depending on the given situation such as area or distance involved and personnel available enumeration of fish kills may be approached in one of the following ways.

1) For large rivers, establish observers at a station or stations (e.g., bridges) and count the dead and/or dying fish for a specified period of time, then project same to total time involved.

2) For large rivers and lakes, traverse a measured distance of shoreline, count the number and kinds of dead or dying fish. Project same relative to total distance of kill.

3) For lakes and large ponds, count the number and species within measured areas, and then project to total area involved.

4) For smaller streams one may walk the entire stretch involved and count observed number of dead individuals by species.

3 Biological sampling Macro-Invertebrates

a Sampling of benthic organisms after the more urgent aspects of the kill investigation has been completed can prove to be rewarding relative to extent and cause of kill. Since this general form of aquatic life is somewhat sedentary by nature any release of deleterious materials to their environment will take its toll. Thus by making a series of collections up and downstream, the affected stretch of stream may be delineated when the benthic populations are compared to those from the control area. Also the causative agent may be realized when the specifics of the benthic population present are analyzed.

b Other aspects of the biota which should be considered are the aquatic plants. In lakes and ponds floating and rooted plants should be enumerated and identified. The collection of plankton (rivers and lakes) should be taken in order to determine the degree of bloom, which in itself may cause fish kills because of diurnal DO levels.

c Both aquatic plants and macro-invertebrates may be preserved in a 5% formalin solution.

4 Bioassay

Static bioassay techniques as outlined in Standard Methods may be effectively used to determine acute toxicity of wastes as well as receiving waters.

a In situ using live boxes

b Mobile bioassay laboratory

c Samples returned to Central Lab for toxicity tests

V DETAILED EXAMINATION OF SOURCE OF POLLUTION

A Seven general categories under which causes of kills can be grouped are:

1 Industrial waste discharges

2 Waste discharges from municipal sewerage systems

3 Water treatment plant discharges

4 Agriculture and related activities

5 Temporary activities

6 Accidental spills of oil and other hazardous substances

7 Natural causes

B Industrial Waste Discharges

1 Upon locating the outfall source, collect a sample immediately if possible at the point where the wastes leave the company property.

2 Make an in-plant inspection if possible.

a Contact the plant manager or person in charge

b Request a brief tour of the facilities

c Obtain general information concerning the products manufactured, raw materials, manufacturing process, quantities, sources, and characteristics of wastes, and waste treatment facilities if any Possibly the company may be able to supply a flow diagram or brochure of the plant operations.

d Request specific information concerning the plant operation immediately prior to the start of the kill

C Waste discharges from a municipal or domestic type sewerage system

1 Discharges from this source may be domestic sewage and industrial wastes combined with domestic sewage. These wastes may be subjected to treatment of a municipal treatment plant or may be discharged directly, untreated to a stream

2 Generally, the municipality or owner of the sewerage system is held responsible for any discharge in such a system, consequently, after collecting samples, the owner or a representative of the owner of the sewerage system should be contacted. This may be a sewage treatment plant operator, city engineer, public works supervisor, a subdivision developer, etc

a Obtain information about the operation of the system

b If the cause of the kill was the result of an industrial waste discharge to a municipal sewer and thence to a stream, information should be obtained from a municipal official about the industry and the problem. An inspection of the industrial plant may be desirable. Generally, this should be done only in cooperation with a municipal official

D Agriculture and Related Activities

1 Pollution capable of causing fish kills may result from such agricultural operations as crop dusting and spraying fertilizer applications, and manure or other organic material discharges to a stream

2 Generally, kills related to these factors will be associated with high rains and runoff.

3 The source or type of pollution may be difficult or impossible to locate exactly. It may involve a large area. Talking to local residents may help pinpoint the specific problem area Runoff from fields, drainage ditches, and small streams leading to the kill area are possible sampling places which may be used to trace the cause

E Temporary Activities

1 Causes of kills may result from such temporary or intermittent activities as mosquito spraying, construction activities involving chemicals, oils, or other toxic

substances, and weed spraying with herbicide containing materials toxic to fish such as arsenic.

2 As with agricultural activities, tracing the cause of these kills is difficult and may require extensive sampling.

3 Accidental spills from ruptured tank cars, pipelines, etc., and dike collapse of industrial ponds are frequently sources of fish kills.

F Possible Natural Causes of Fish Kills

1 Types of natural causes

a Oxygen depletion due to ice and snow cover on surface waters

b Oxygen depletion at night because of plant respiration or at any-time during the day because of natural occurring organics in the water

c Abrupt temperature changes

d Epidemic and endemic diseases, parasites, and other natural occurring biological causes

e Lake water inversion during vernal or autumnal turnover which results in toxic material or oxygen-free water being brought to the surface

f Interval seiche movement in which a toxic or low DO hypolimnion flows up into a bay or bayou for a limited period of time, and later returns to normal level

2 Fish kills in rivers below high dams immediately following the opening of a gate permitting hypolimnionic water to flow down the stream (as in TVA region)

VI CASE HISTORY

A The Lower Mississippi Endrin kill is an excellent example of the investigation of a major fish kill Bartsch and Ingram give the following summary (See Table 1).

TABLE 1

ELEMENTS OF INVESTIGATIONS

I Examination of usual environmental factors

II Elimination of parasites, bacterial or viral diseases and botulism as causes of mortalities*

III Considerations of toxic substances Examination and prognostication of symptoms of dying fish Autopsy, including

- Haematocrits and white cell counts
- Kidney tissue study
- Brain tissue assay for organic phosphorus insecticide
- Tissue analysis for 19 potentially toxic metals
- Gas chromatographic analysis of tissues, including blood, for chlorinated hydrocarbon insecticides

IV Explorations for toxic substances

- Bioassay with Mississippi River water
- Bioassay with extracts from river bottom mud
- Bioassay with tissue extracts from fish dying in river water and bottom mud extracts
- Bioassay with endrin to compare symptoms and tissue extract analyses with those of dying fish in all bioassays

V Intensive chemical analysis for pesticides in the natural environment, experimental environment, river fish, and experimental animals

VI Surveillance of surface waters for geographic range and intensity of pesticide contamination

VII Correlation and interrelation of findings

* The investigator should be aware of the fact that apparently healthy fish may be harboring pathogenic bacteria in their bloodstreams (see Bullock and Snieszko) Thus there may be several factors involved in fish mortalities, all of which may obscure the primary cause or causes

The investigation was designed to consider and eliminate potential fish kill possibilities that were not involved and come to a point focus on the real cause. It was found that the massive kills were not caused by disease, heavy metals, organic phosphorus compounds, lack of dissolved oxygen or unsuitable pH. Blood of dying river fish was found to have concentrations of endrin equal to or greater than laboratory fish killed with this pesticide, while living fish had lesser concentrations. Symptoms of both groups of dying fish were identical. It was concluded from all data obtained that these fish kills were caused by endrin poisoning.

B Recent investigations in Tennessee have shown that the leaking of small amounts of very toxic chemicals from spent pesticide-containing barrels used as floats for piers and diving rafts in lakes and reservoirs can produce extensive fish kills. The particular compound used to control slime growth in manufacturing processes, contained two primary chemicals in solution (phenylmercuric acetate and 2, 4, 6-trichlorophenol) The former compound which breaks down to form diphenylmercury was found to be more toxic to aquatic life than the latter.

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This outline was prepared by Jack Geckler, Research Aquatic Biologist, Fish Toxicology Activities, EPA, Newtown, OH 45244

Project Personnel Contacted

a. Name ______

b. Means of Contact ______

c. Date & Time ______

1. Reporting source

   a. Agency ______

      (1) Address ______

      (2) Phone (s) ______

   b. Individual ______

      (1) Address ______

      (2) Phone ______

      (3) Fish Kill Network ______ yes ______ no

   c. Other Contacts ______

      (1) Address ______

      (2) Phone ______

      (3) Fish Kill Network ______ yes ______ no

2. Data furnished by reporting source

   a. Location of Kill ______

   b. Dates of Kill ______ Dying last observed ______

   c. Kinds of organisms ______

   d. Approximate number killed ______

   e. Cause of kill (if known) ______

   f. Suspected causative sources ______

   g. Measures taken ______

   h. Other Agencies contacted ______

      (1) Date and Time ______

3. Action requested

   a. Field investigations ______

   b. Laboratory analysis ______

4. Assistance to Project

   a. Provided by ______

   b Personnel ______

   c. Equipment ______

   d. Transportation facilities ______

AN INITIATION INTO STATISTICS

I INTRODUCTION

Quantitative statistical analysis has been publicized by some analyst as a cure all. This exaggerated claim is adhered to by only a few opportunists.

Quantitative analysis should be thought of as a valuable tool which can be exploited by close cooperation between the statistician and the investigator in a joint attempt to find better methods of controlling or understanding the complex interaction of men, materials, machines and the natural environmental resources.

Modern statistics is still a relatively new subject with along list of unsolved statistical problems. Unless caution is observed the inevitable result will be some unhappy experiences with inadequately equipped amateurs. In point of fact, most statistical procedures are quite simple and intuitively acceptable once they have been pointed out in a particular context. Heuristic persuasion can be effectively used to give insights into the concept, development and foundations of the particular tool used.

II IMPORTANCE OF PREPLANNING

Many investigators now believe that statistics and particularly the statistical design of data collection has potentially impressive leverage effects on the amount of information to be included and obtainable from any research effort.

After all, any finite body of data contains only a limited amount of information. This limit is set by the inherent nature of the data themselves and cannot be increased by any amount of ingenuity exercised by the data analyst. However intelligent preplanning can increase this limit.

Statistical methods cannot reveal anything that is not already implicit in the data. However, every correct inference of interest possible should be made. A continual effort should be made to recover all of the information but there never will be a way to find more than is already there.

It would be better to do enough work on five projects than an inadequate amount on a much larger number. You don't have to plan in order to fail, all you have to do is fail to plan.

Many investigators still believe that statisticians only enter at the end of the work--that is, at the final examination of the data. It is small comfort to the investigator to be told after the event, that the answers to his questions are inconclusive and were bound to be so because an inadequate amount of effort was allotted to the work.

It is well known that the end doesn't justify the means but in data collection and analysis the end dictates the means. The importance of preplanning cannot be overemphasized. If you fail to plan then you are planning to fail.

Any investigation problem can be viewed as a five step process. These are:

1 Problem definition

2 Data collection scheme

3 Actual data collection

4 Data analysis

5 Written report or recommendations

The most important step is the first one because the second is based upon it. The third follows from the second, etc. The process is like a chain with five links. If the first link is incorrect, the entire chain is. Too often, too little time is spent on step one. Many times the problem is vaguely or ill defined. The best protection against these faults is to write down black on white a statement of the problem and have all connected with the investigation to agree on the statement. With this as an introduction we proceed to describe the field of statistics briefly.

III ALTERNATIVE VIEWS OF STATISTICS

The field of statistic can be described from many different angles. Each viewpoint results in a different insight into this vast discipline. In this introduction we consider the entire field from three viewpoints. The first view is very simple, the second introduces more details and finally the last viewpoint is very comprehensive with respect to subject matter but is only one molecule deep.

IV FIRST VIEWPOINT

The first way of looking at statistics is to partition the data source into the method of acquisition as either experimental or nonexperimental.

FIGURE 1

FOURFOLD MAP OF ENDS AND MEANS IN APPLIED STATISTICS

PURPOSE OR ENDS OF STATISTICS	Source Or Means Of Data Acquisition: Nonexperimental	Source Or Means Of Data Acquisition: Experimental
Enumerative or Descriptive Answers: question, how much Aim: adjustment to the environment	Tables and graphs Averages central tendency Dispersion measures Frequency curves Correlation Survey sampling Index numbers	) Same (for Tables and graphs, Averages central tendency, Dispersion measures, Frequency curves, Correlation) Assessment of probabilities Theoretical means, variances, etc. and corresponding confidence intervals
Explanatory or analytic causal inference Answers: question, why Aim: ascendancy over the environment	Specialized branches of applied statistics Demography Econometrics Genetics Causal inference from time series	Design of factorial experiments Randomization methods Significance tests Regression analysis Analysis of variance and covariance Probit analysis

In an experimental investigation, control is exercised over some or all of the influencing variables, while in a nonexperimental investigation nature is merely measured as is without any control on any of the influencing variables.

The purpose of the analysis is also viewed as two-fold, that is, either enumerative or analytic. The combination of double means and ends leads to the four-fold table in Figure 1 This is taken essentially from reference 1.

Historically, the field of statistics started with the upper left quadrant about the turn of the century. The research proceeded clockwise until the lower left hand quadrant was reached. That is causal inference from observational data. This quadrant is the most difficult and has been receiving the most research attention lately.

In each quadrant some of the techniques are indicated and the reader should be further cautioned that the lines drawn are fluid and are not rigid. A partial listing has been given in each quadrant and no attempt has been made to give a 100% enumeration.

V SECOND VIEWPOINT

The next viewpoint of statistics is in this orientation the one taken by The International Statistical Institute Figure 2 shows their scheme for classification of abstracts Any article in statistics can be classified in one or more of the indicated titles. Some articles are given a primary classification and others in addition are given a secondary classification.

Let us give a brief introduction into the major topics listed in Figure 2.

Upon inspection of the subtitles of the first topic, mathematical methods, it is evident that these subtitles are a list of the mathematical techniques used by the professionals to solve the difficult problems encountered in the development of the theory and methods.

FIGURE 2

SCHEME FOR CLASSIFICATION OF ABSTRACTS

0. MATHEMATICAL METHODS (White)
   0. General papers
   1. Solution of equations
   2. Methods of curve and surface fitting, smoothing
   3. Interpolation and quadrature
   4. Special functions and transforms
   5. Functional relationships
   6. Determinantal and matrix analysis
   7. Game theory
   8. Programming techniques
   9. Group and field theory
   10. Graph theory and combinatorial analysis
   11. Measure theory
   12. Optimisation

1. PROBABILITY (Pink)
   0. General papers
   1. Calculus of probabilities
   2. Expected values
   3. Combinatorial problems
   4. Geometric probability
   5. Limit theorems
   6. Stochastic convergence
   7. Stochastic approximation
   8. Decision theory and functions
   9. Transforms Fourier, Laplace, etc.
   10. Convolutions

2. FREQUENCY DISTRIBUTIONS (Green)
   0. General papers
   1. Descriptive properties
   2. Transformations of variates
   3. Normal and lognormal
   4. Binomial, multinomial and hypergeometric
   5. Poisson, exponential, negative binomial, logarithmic and contagious
   6. Rectangular, extreme value and Weibull
   7. Pearson and "series expansion" distributions
   8. Truncated and mixed distributions
   9. Multivariate distributions
   10. Limit distributions
   11. Approximations
   12. Other distributions

FIGURE 2 (continued)

3. SAMPLING DISTRIBUTIONS (Light Blue)
   0. General papers
   1. t, z, F and x^2 distributions
   2. Non-central distributions
   3. Studentisation
   4. Quadratic forms
   5. Correlation and regression coefficients
   6. Location and scale statistics
   7. Shape and other descriptive statistics
   8. Order statistics
   9. Multivariate problems
   10. Limit distributions
   11. Linear forms

4. ESTIMATION (Yellow)
   0. General papers
   1. Properties of estimators
   2. Types of estimator Bayes, maximum likelihood, least squares, etc.
   3. Individual estimators point
   4. Individual estimators: interval
   5. Inequalities tolerance limits and regions
   6. Distribution-free methods
   7. Sequential methods
   8. Multivariate problems
   9. Finite population procedures - surveys
   10. Simultaneous estimation
   11. Distribution functions and densities
   12. Decision theory

5. HYPOTHESIS TESTING (Purple)
   0. General papers
   1. Properties of test
   2. Individual hypotheses
   3. Two-sample problem
   4. k-sample problem
   5. Outliers
   6. Distribution-free tests
   7. Sequential tests
   8. Multivariate problems
   9. Types of test· likelihood ratio, Bayes, minimax, etc.
   10. Goodness-of-fit tests
   11. Combining and comparing tests
   12. Decision theory

6. RELATIONSHIPS (Grey)
   0. General papers
   1. Regression, linear hypothesis, polynomials
   2. Correlation inc. canonical correlation
   3. Factor methods and principal components
   4. Discriminant analysis and cluster analysis
   5. Ranking and scaling methods
   6. Systems of equations· structure
   7. Non-linear equations-logistic
   8. Transformed relationships-quantal response
   9. Association and contingency
   10. Functional relationships
   11. Non-standard conditions
   12. Other multivariate methods

7. VARIANCE ANALYSIS (Biscuit)
   0. General papers
   1. Fixed effects model
   2. Variance components model
   3. Mixed and other models
   4. Non-orthogonal data and missing values
   5. Non-standard conditions-failure of assumptions
   6. Covariance analysis
   7. Multiple comparisons, multiple decision procedures
   8. Ranked data
   9. Sequential methods inc. preliminary tests
   10. Combining sets of results
   11. Precision of measurement
   12. Multivariate models

8. SAMPLING DESIGN (Orange)
   0. General papers
   1. Simple random, stratified, multi-stage
   2. Sampling with unequal probability
   3. Multi-phase sampling, double sampling
   4. Natural (human, animal and biological) populations
   5. Non-sampling problems
   6. Censored, systematic and quota sampling
   7. Nature and number of units, cost and efficiency
   8. Acceptance inspection
   9. Process control

FIGURE 2 (continued)

9. DESIGN OF EXPERIMENTS (Blue)

0. General papers
1. Block designs, designs for two-way elimination of heterogeneity
2. Factorial arrangements
3. Response surfaces
4. Nature of unit, number of replications, cost and efficiency
5. Paired comparisons and matching problems
6. Preference tests
7. Repeated and sequential experiments
8. Weighing problems
9. Sensitivity problems
10. Systematic designs
11. Screening tests
12. Other designs, e.g., mixtures

10. STOCHASTIC PROCESSES AND TIME SERIES (Red)

0. General papers
1. Properties of individual process
2. Estimation problems
3. Tests of hypotheses
4. Queueing, storage, risk and congestion theory
5. Information theory
6. Stationary processes and spectral analysis
7. Auto and serial correlation
8. Multivariate processes
9. Biological population studies, genetic models
10. Renewal theory
11. Markov processes
12. Branching processes

11. MISCELLANEOUS AND SPECIAL TOPICS (Cream)

0. General statistical methodology
1. Statistical tables and charts
2. Probability graph papers
3. Nomograms and graphic methods
4. Machine methods, hand and punched cards
5. Machine methods, electronic digital
6. Machine methods, other
7. Monte Carlo methods
8. Index numbers
9. History, biography and bibliography
10. Inventory
11. Life-testing and reliability
12. Teaching and training methods

These are the tools used to extend the present boundaries of knowledge. They are not statistics per se but are an indispensable aid to the research statistician.

The next topic, probability, can be described as a method for quantitizing uncertainty where the limits of uncertainty are a numerical value which does not lie outside the range from zero to one. Most people have a correct intuitive feel for probability. If an event can happen (i. e., it will rain) say with probability 0.9 (or 90%) then one knows that the event could or could not occur. However, for a large number of trials, on the average nine times out of ten it will occur (or rain) and one in ten it won't.

There is no universally acceptable abstract calculus of probability. That is to say if one adopted a set of axioms and developed the theory of probability then the inevitable result would be some controversy and many heated arguments. Some fault would be found in the development of the theory by the purists. The purists are those who are concerned with the philosophical and correct logical foundations. They are necessary and serve a useful purpose. They are not boat rockers but keep it from sinking because they plug any holes that may be present.

Most statisticians view probability from a practical viewpoint and their attitude is that it works and does a good job, so let the purists thrash out the difficulties.

In the third topic, frequency distribution, studies are concerned with characterizing and identifying the distribution of a variable or measurable characteristic. The most famous of these distribution is the bell shaped symmetric curve known as the normal distribution. It is characterized by two parameters, namely, the mean, a measure of central tendency and the standard deviation, a measure of spread or variability.

The list of distribution used in statistic is greater than the subtitles listed. This list includes only the most important ones and a complete list of distributions developed by researchers with their properties would fill a book.

The usefulness of a frequency distribution lies in the fact that from it one can calculate the probability of occurrence of different values or set of values for the variable.

Very few people have the luxury when doing research of measuring every unit in the population. For this reason sampling distribution per se are studied quite vigorously. Constraints of time, money, people and resources needed, versus available, force researchers into taking a sample or a subset of the entire population. On the basis of a sample, decision statements or estimates are made about the entire population. Different samples have different values or a sample varies from one sample set to another. Each sample as well as any value calculated from it has its own sampling distribution. These must be known before quantitative statements can be made.

The three most important sampling distributions are the t, F, and Chi-square. They, as well as the normal, are tabulated in almost every textbook on statistics.

In the fifth subject, estimation, the sample values are used to infer information about the entire population. For example, from a sample, one can calculate the sample mean and use this to make statements about the parent population mean. The calculated sample mean ($\bar{X}$) is an estimate of the population mean (the symbol used is the Greek letter mu).

One legitimate question that can be asked is "Is this the best possible way?" Best possible way is a fuzzy concept and must be unfuzzied before the question can be answered. That procedure produces many interesting paths as evidenced by the list of subtitles.

Hypothesis testing (or significance testing), our next topic, is a technique that is too often not really understood. It can be understood by anyone since the concept is really quite simple and is analogous to a comparison procedure familiar to everyone. A test of hypothesis is the familiar method of comparing two things (an unknown and a known) for identity or nonidentity. After the comparison has been completed then one of two conclusions is made: 1) the two things compared are not identical or 2) the things compared are identical.

In the world of statistics, the unknown in the comparison procedure is the sampled population. The known used for the comparison is a hypothetical population which is also called the mathematical model or the test assumptions.

The sampled population values are used to evaluate a statistic which can be thought of as a formula for combining the information available in the sample. The observed statistic value obtained from the sample is compared with corresponding values obtained from the mathematical model.

If the observed and expected statistic values are in disagreement then the sampled and hypothetical population disagree or are not identical. If the statistic values agree the populations agree or are identical.

A simple example will make the ideas clear. Assume you are sampling from normal with an unknown mean and a standard deviation equal to ten. We wish to test if the unknown mean equal five. The mathematical model or known is normal, mean = 5 and standard deviation = 10. If the conclusion of the comparison procedure is agreement between the sampled population and the mathematical model. This means both normals have equal means and standard deviations. That is equivalent to saying the two means are equal to five or the unknown mean from the sampled population equals five. If the statistical decision was the two populations disagree then the evidence says that the mean of the sampled population doesn't equal five.

In summary, a one sentence nontechnical definition for a test of hypothesis could be "A test of hypothesis can be defined as a method for comparing an unknown sampled population with a known population (or mathematical model) and deciding if the two populations are in agreement (or are alike) or disagreement (or are not alike)."

A longer nontechnical working definition for hypothesis testing would be as follows. A test of significance can be defined as a method of analyzing data so as to discriminate between two hypotheses. The first hypothesis is called the null hypothesis which means there is no difference between sampled and hypothetical population. The difference between the two populations is equal to zero or is null. The second hypothesis is called the alternative hypothesis which should be the operational statement of the experimenters research hypothesis. Next the best test for the alternative hypothesis is selected, that is, the one that has the highest probability of saying the research hypothesis is true when it is in fact true. The probability of deciding the null hypothesis is false when true is selected by the researcher. This value is called the alpha-error. The alpha-error specifies those values for the statistic for which the decision is agreement (or do not reject H_0 or there is no difference between sampled and hypothetical populations) or there is disagreement (accept alternative or research hypothesis). The sample is now calculated and the statistic value is compared with the decisions values determined by the alpha-error. The decision is made to either: 1) do not reject the null hypothesis or, 2) reject the null hypothesis (or equivalently accept research hypothesis).

For those readers who want an in depth nontechnical explanation of a tests of significance, the references listed by this writer are suggested as a beginning.

Our next topic relationship needs no introduction since every scientist understands that some sort of predictive technique is implied.

Variance analysis is next on our list. In variance analysis, the total variation displayed by a set of observations may, in certain circumstances be separated into components associated with defined sources of variation. The defined sources are used as a criterion of classification for the observations. That portion of variance which is left undefined or explained is called experimental error. Many standard situations can be reduced to the variance analysis form.

Our ninth topic has many aliases, two examples are sampling plan and survey design. The purpose is to measure nature as it exists by taking a sample from a population. Statistical data are collected to provide a rational basis for action. The action may call for the enumerative interpretation of the data or it may call for an analytic interpretation. These actions determine the difference between enumerative and analytic surveys. Enumerative surveys would be classified in the upper left hand quadrant of Figure 1, while analytic surveys should appear in the lower left quadrant of that same figure.

In a design of experiment, our next topic, the researcher is interested in the change produced in a response by the influencing factors. For example, a researcher may be interested in the yield in bushels of wheat (the response) when different fertilizers are used in different amounts (the influencing factors under his control). The design of experiment consists of the set of rules which match treatments (or list of influencing factors to be studied) with the units (plots of ground) to which the treatments are to be applied. The principles of randomization, replication and stratification are employed and produce the many designs available.

In the eleventh topics, the adjective stochastic implies the presence of a random variable. Hence a stochastic process is one wherein the system incorporates an element of randomness as opposed to a deterministic system. A time series is a set of ordered observations on a measurable characteristic taken at different points in time.

The final classification is the miscellaneous basket for topics which are not large enough for their own major classification.

VI THIRD VIEWPOINT

The final viewpoint will be the question and answer approach. This will lead to a more detailed problem classification viewpoint and tie in some of the topics discussed in the second viewpoint.

Suppose you are interested in the B.O.D. measurements at a particular point in the Bay of San Francisco. The totality of all such measurements will be called a parent population and will be represented by some distribution as shown in Figure 3.

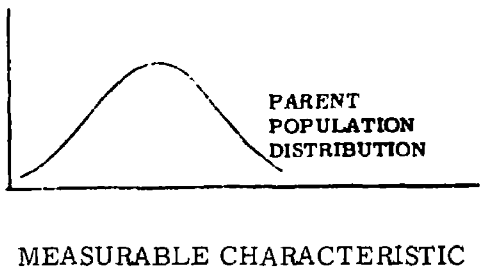

Figure 3

Three questions the researcher can ask at this point are:

1) What is the research hypothesis? Does my boss agree?

2) What is the definition of our population?

3) Am I going to study my population as is or subdivide it and study each subdivision?

Let us assume that the answer to the first question in our imaginary study has been finalized. Question one is still the best starting point in any investigation.

In this imaginary study, the answer to the second question is clearly answered but in other studies, the question is not always easily answered.

Some answers to the third question could be as follows: I will subdivide the population and compare daytime versus nighttime. An alternative choice could be, I will subdivide into the four seasons and compare.

After these three questions have been satisfactorily answered, the next question could be:

4) Will I be able to measure the entire population or take a sample?

If the answer to this question is: I will measure all the population, then the data analysis will be descriptive statistics. Statistical analysis is subdivided into the two main areas, namely descriptive and inferential statistics.

The first of these is descriptive statistics which is concerned with the problem of how you condense a large mass of data in such a way so that you summarize the available data into several succinct numbers which is simple to interpret and easier for the mind to grasp and comprehend. This type of statistic is the one students first meet in a course in statistics (See Figure 4).

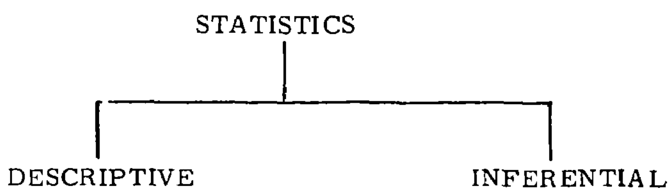

FIGURE 4

If the answer to question 4 is take a sample (few investigators have the luxury of measuring the entire population) then several other good questions occur like the following

5) How big a sample do I need? Is my budget adequate for it?

6) If sample size is not fixed in advance, how will data collection be terminated?

7) How will our samples be obtained? Time, place, order, etc.

8) If our samples are not random, how will we generalize?

Statistical theory assumes random samples, hence violation of this assumption invalidates the application of the theory.

9) Am I going to use the sample for statistical inference with respect to estimation or hypothesis testing? (See Figures 5 and 6).

Hypothesis testing can be briefly defined as a method of analyzing data so as to discriminate between two hypotheses. The one hypothesis is the research hypothesis while the other is generally the negation of the research hypothesis.

Estimation is concerned with statements about the numerical value of unknown population parameters from sample data. If the estimate is a single number, then it is called a point estimate. If an interval is calculated within which the parameter lies in a probability sense, then it is called an interval estimate. (See Figure 7)

Hypothesis testing can be subdivided into two broad types.

If the parent population is normally distributed, then the hypothesis testing is said to be parametric. If normality cannot be proven or safely assumed, then the test is said to be nonparametric. (See Figure 7)

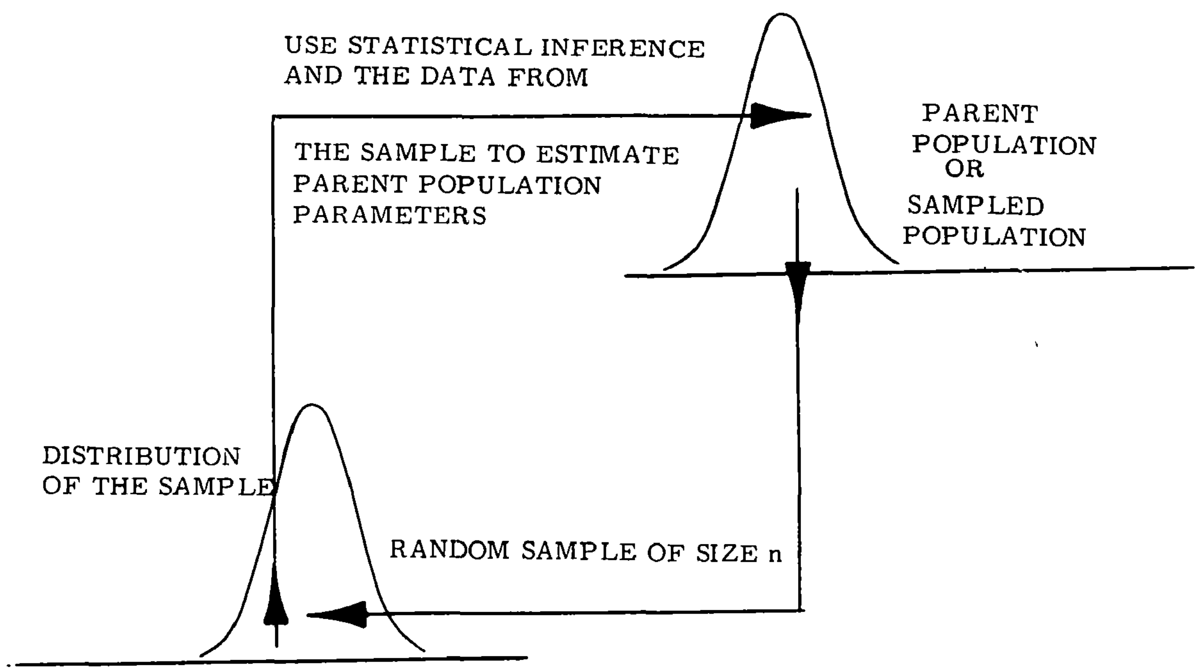

FIGURE 5

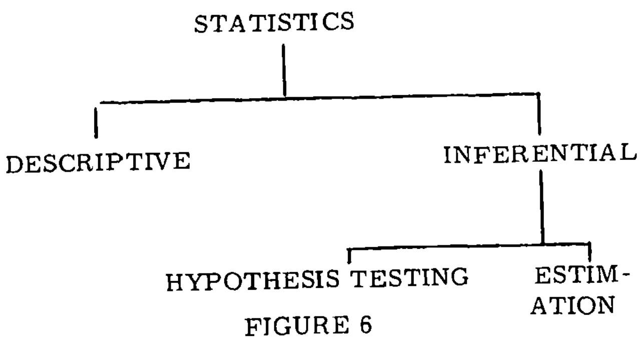

FIGURE 6

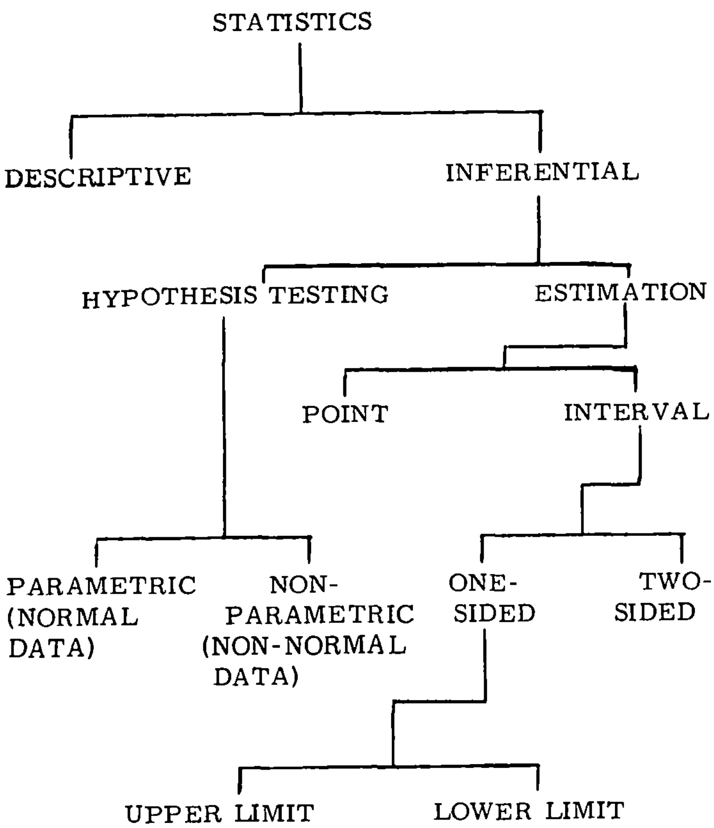

FIGURE 7

If hypothesis testing is to be applied, then some pertinent questions are:

10) What is the null hypothesis?

11) What is the alternative hypothesis?

12) What is the Type I error?

13) What is the Type II error?

14) What is the consequence of a Type I error?

15) What is the consequence of a Type II error?

At this point, other thought provoking questions can be asked.

16) What are the variables not measured that affect my observations?

17) Are we going to reject observations that seem aberrant? If so, on what basis, objective or subjective?

18) Have I given any thought to lost information?

19) Have I given any thought to precision, accuracy and bias of measurements?

20) If there is more than one observer involved, how will I insure uniformity or separate their differences as it affects my results.

21) How will we minimize our nonsampling errors?

Some more thought-provoking questions of general nature are

22) Will the variable observed be the one I am really interested in?

23) Is the data analysis predetermined before data collection or will this be determined after data collection?

24) What is it we are trying to do?

25) What do I have to work with?

26) What do I need to know?

27) Who else is concerned or should be?

I would like to close this section by asking some pointed questions related to the computer.

28) Is a computer going to be used or will the analyses be manual?

29) If a computer is going to be used, are the programs needed available?

30) If the computer programs are not available now, will they be ready when the data has been collected?

31) If the computer is going to be used and a large mass of data will have to be entered, how will the data be fed into the computer?

VII RECOMMENDATIONS

There are many short courses in statistics available through EPA National Training Center. Figures 8 and 9 list the 5-day courses and the 8-hour seminars dealing with statistics.

FIGURE 8

5-DAY	
Number	Title
801	Basic Environmental Statistics
	Environmental Statistics
802	Design of Experiments
804	Nonparametric
806	Analyzing Qualitative Data
810	Applied Regression Analysis
815	Sample Size Determination
820	Survey Sampling

FIGURE 9

8-Hour Seminars	
Number	Title
899.1	Survey Sampling for Managers
899.2	Analysis of Variance and Design of Experiments
899.3	Regression and Correlation
899.4	An Introduction to Hypothesis Testing
899.5	Statistical Quality Control
899.6	Estimation and Hypothesis Testing for Normal
899.7	Nonparametric Hypothesis Testing
899.8	Introduction to and the Analysis of Categorical Data
899.9	An Introduction to Probability

The five-day courses are concerned with applications while the seminars are designed to acquaint the listeners with the topics and the type problems that can be solved.

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This outline was prepared by Mr. J. F. Santner, Mathematical Statistician, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

USING BENTHIC BIOTA IN WATER QUALITY EVALUATIONS

I BENTHOS ARE ORGANISMS GROWING ON OR ASSOCIATED PRINCIPALLY WITH THE BOTTOM OF WATERWAYS

Benthos is the noun.

Benthonic, benthal and benthic are adjectives.

II THE BENTHIC COMMUNITY

A Composed of a wide variety of life forms that are related because they occupy "common ground"--the waterways bottom substrates. Usually they are attached or have relatively weak powers of locomotion. These life forms are

1 Bacteria

A wide variety of decomposers work on organic materials, breaking them down to elemental or simple compounds (heterotrophic). Other forms grow on basic nutrient compounds or form more complex chemical compounds (autotrophic).

2 Algae

Single-cell plants that are the basic producers of food that nurtures the animal components of the community.

3 Flowering Aquatic Plants (Pondweeds)

The largest flora, composed of complex and differentiated tissues. Many are rooted.

4 Micro-Fauna

Animals that pass through a U.S. Standard Series No. 30 sieve, but are retained on a No. 100 sieve. Examples are rotifers and microcrustaceans. Some forms have organs for attachment to substrates, while others burrow into soft materials or occupy the interstices between rocks, floral or faunal materials.

5 Macro-Fauna

Animals that are retained on a No. 30 sieve. This group includes the insects, worms, molluscs, and occasionally fish. Fish are not normally considered as benthos, though there are bottom dwellers such as sculpins and darters.

B It is a self-contained community, though there is interchange with other communities. For example: Plankton settles to it, fish prey on it and lay their eggs there, terrestrial detritus is added to it, and many aquatic insects migrate from it to the terrestrial environment for their mating cycles.

C It is a stationary water quality monitor The low motility of the biotic components requires that they "live with" the quality changes of the over-passing waters. Changes imposed in the long-lived components remain visible for extended periods, even after the cause has been eliminated. Only time will allow a cure for the community by migration and reproduction

III HISTORY OF BENTHIC OBSERVATIONS

A Ancient literature records the vermin associated with fouled waters.

B 500-year-old fishing literature refers to animal forms that are fish food and used as bait.

C The scientific literature associating biota to water pollution problems is over 100 years old (Mackenthun and Ingram, 1964).

D Early this century, applied biological investigations were initiated.

1 The entrance of State boards of Health into water pollution control activities.

2 Creation of state conservation agencies.

3 Industrialization and urbanization.

4 Growth of limnological programs at universities.

E A decided increase in benthic studies occurred in the 1950 decade, and much of today's activities are strongly influenced by developmental work conducted during this period. Some of the reasons for this are:

1 Movement of the universities from "academic biology" to applied pollution programs.

2 Entrance of the federal government into enforcement aspects of water pollution control.

3 A rising economy and the development of federal grant systems.

4 Environmental Protection Programs are a current stimulus.

IV WHY THE BENTHOS?

A It is a natural monitor

B The community contains all of the components of an ecosystem.

1 Reducers

2 Producers

3 Consumers

a Herbivores

b Predators

C Economy of Survey

1 Manpower

2 Time

3 Equipment

D Extensive Supporting Literature

V REACTIONS OF THE COMMUNITY TO POLLUTANTS

A Destruction of Organism Types

1 Beginning with the most sensitive forms, pollutants kill in order of sensitivity until the most tolerant form is the last survivor. This results in a reduction of variety or diversity of organisms.

2 The usual order of macroinvertebrate disappearance on a sensitivity scale below pollution sources is shown in Figure 2.

Stoneflies
Mayflies
Caddisflies
Amphipods
Isopods
Midges
Oligochaetes

As water quality improves, these reappear in the same order.

B The Number of Survivors Increase

1 Competition and predation are reduced between forms.

2 When the pollutant is a food (plants, fertilizers, animals, organic materials).

C The Number of Survivors Decrease

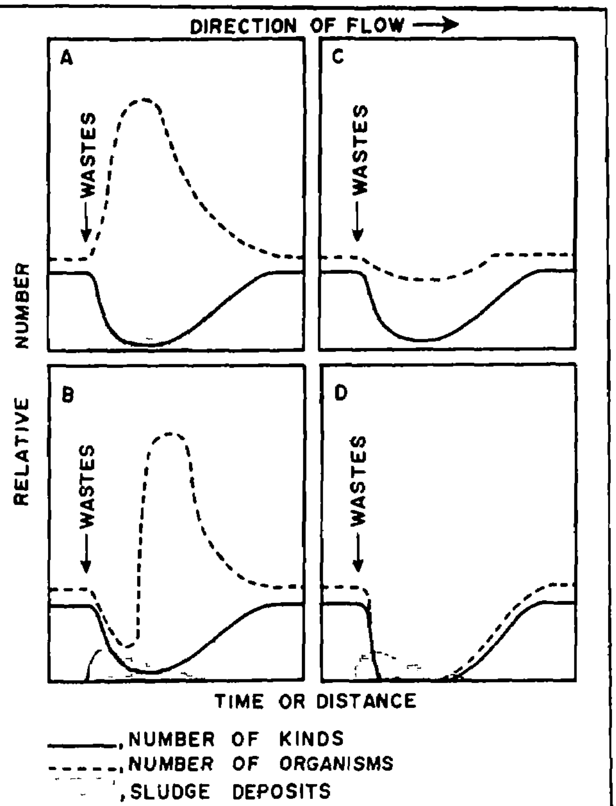
1 The material added is toxic or has no food value.

2 The material added produces toxic conditions as a byproduct of decomposition (e.g., large organic loadings produce an anaerobic environment resulting in the production of toxic sulfides, methanes, etc.)

D The Effects May be Manifest in Combinations

1 Of pollutants and their effects.

2 Vary with longitudinal distribution in a stream. (Figure 1)



Four basic responses of bottom animals to pollution A Organic wastes eliminate the sensitive bottom animals and provide food in the form of sludges for the surviving tolerant forms B Large quantities of decomposing organic wastes eliminate sensitive bottom animals and the excessive quantities of byproducts of organic decomposition inhibit the tolerant forms; in time, with natural stream purification, water quality improves so that the tolerant forms can flourish, utilizing the sludges as food C. Toxic materials eliminate the sensitive bottom animals, sludge is absent and food is restricted to that naturally occurring in the stream, which limits the number of tolerant surviving forms Very toxic materials may eliminate all organisms below a waste source D. Organic sludges with toxic materials reduce the number of kinds by eliminating sensitive forms Tolerant survivors do not utilize the organic sludges because the toxicity restricts their growth

Figure 1

E Tolerance Grouping (Figure 2)

Flexibility must be maintained in the establishment of tolerance lists based on the response of organisms to the environment because of complex relationships among varying environmental conditions. Some general tolerance patterns can be established. Stonefly nymphs, mayfly naiads, hellgrammites, and caddisfly larvae represent a grouping (sensitive or intolerant) that is quite sensitive to environmental changes. Blackfly larvae, scuds, sowbugs, snails, fingernail clams, dragonfly nymphs, damselfly nymphs, and most kinds of midge larvae are intermediate (facultative or intermediate) in tolerance. Sludgeworms, some kinds of midge larvae (bloodworms), and some leeches are tolerant to comparatively heavy loads of organic pollutants. Sewage mosquitoes and rat-tailed maggots are tolerant of anaerobic environments.

F Structural Limitations

The morphological structure of a species limits the type of environment it may occupy. Species with complex appendages and exposed, complicated respiratory structures, such as stonefly nymphs, mayfly numphs, and caddisfly larvae, that are subjected to a constant deluge of settleable particulate matter soon abandon the polluted area because of the constant preening required to maintain mobility or respiratory functions, otherwise, they are soon smothered.

Species without complicated external structures, such as bloodworms and sludgeworms, are not so limited in adaptability. A sludgeworm, for example, can burrow in a deluge of particulate organic matter and flourish on the abundance of "manna." Morphology also determines the species that are found in riffles, on vegetation, on the bottom of pools, or in bottom deposits.

VI SAMPLING PROCEDURES

A Fauna

1 Qualitative sampling determines the variety of species occupying an area. Samples may be taken by any method that will capture representatives of the species present. Collections from such samplings indicate changes in the environment, but generally do not accurately reflect the degree of change. Mayflies, for example, may be reduced from 100 to 1 per square foot. Qualitative data would indicate the presence of both species, but might not necessarily delineate the change in predominance from mayflies to sludge-worms.

2 Quantitative sampling is performed to observe changes in predominance. The most common quantitative sampling tools are the Petersen and Ekman dredges and the Surber stream bottom or square-foot sampler. Of these, the Petersen dredge samples the widest variety of substrates. The Ekman dredge is limited to fine-textured and soft substrates, such as silt and sludge. The Surber sampler is designed for sampling riffle areas; it requires moving water to transport dislodged organisms into its net and is limited to depths of two feet or less.

3 The collected sample is screened with a standard sieve to concentrate the organisms, these are sorted from the retained material, and the number of each kind determined. Data are then adjusted to number per unit area, usually to the number per square foot of bottom or occasionally to number per square meter. This adjustment standardized the method of data expression.

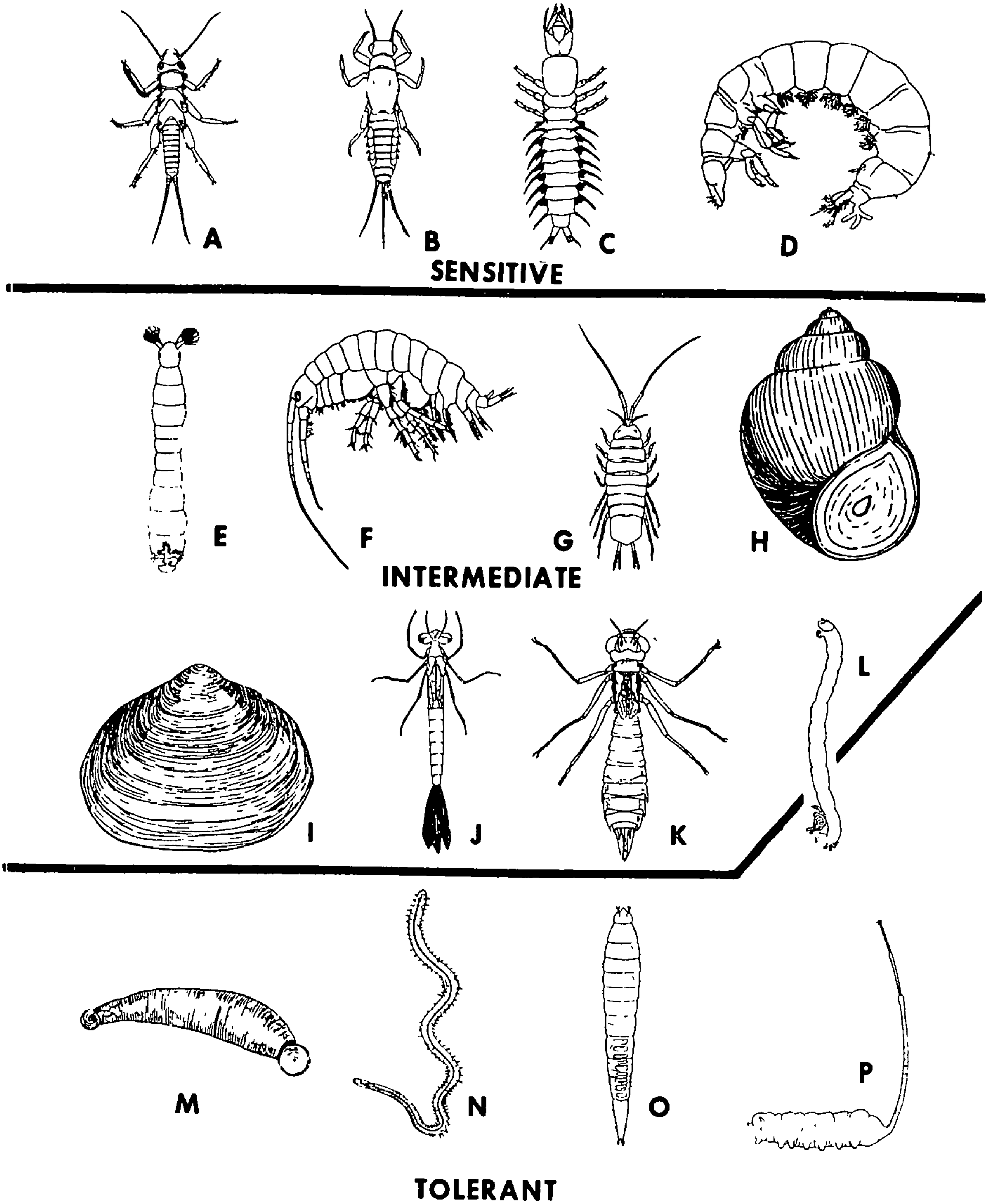
4 Independently, neither qualitative nor quantitative data suffice for thorough analyses of environmental conditions. A cursory examination to detect damage may be made with either method, but a combination of the two gives a more precise determination. If a choice must be made, quantitative sampling would be best, because it incorporates a partial qualitative sample.

REPRESENTATIVE BOTTOM-DWELLING MACROANIMALS

Drawings from Geckler, J., K. M. Mackenthun and W. M. Ingram, 1963. Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control, DHEW, PHS, Cincinnati, Ohio, Pub. No. 999-WP-2.

A	Stonefly nymph	(Plecoptera)	I	Fingernail clam	(Sphaeriidae)
B	Mayfly nymph	(Ephemeroptera)	J	Damselfly nymph	(Zygoptera)
C	Hellgrammite or Dobsonfly larvae	(Megaloptera)	K	Dragonfly nymph	(Anisoptera)
D	Caddisfly larvae	(Trichoptera)	L	Bloodworm or midge fly larvae	(Chironomidae)
E	Black fly larvae	(Simuliidae)	M	Leech	(Hirudinea)
F	Scud	(Amphipoda)	N	Sludgeworm	(Tubificidae)
G	Aquatic sowbug	(Isopoda)	O	Sewage fly larvae	(Psychodidae)
H	Snail	(Gastropoda)	P	Rat-tailed maggot	(Tubifera-Eristalis)

KEY TO FIGURE 2


A
B
C
D
SENSITIVE
E
F
G
H
INTERMEDIATE
I
J
K
L
M
N
O
P
TOLERANT

B Flora

1 Direct quantitative sampling of naturally growing bottom algae is difficult. It is basically one of collecting algae from a standard or uniform area of the bottom substrates without disturbing the delicate growths and thereby distort the sample. Indirect quantitative sampling is the best available method.

Artificial substrates, such as wood blocks, glass or plexiglass slides, bricks, etc., are placed in a stream. Bottom-attached algae will grow on these artificial substrates. After two or more weeks, the artificial substrates are removed for analysis. Algal growths are scraped from the substrates and the quantity measured.

Since the exposed substrate area and exposure periods are equal at all of the sampling sites, differences in the quantity of algae can be related to changes in the quality of water flowing over the substrates.

2 The quantity of algae on artificial substrates can be measured in several ways. Microscopic counts of algal cells (Cooke, 1958, Gumtow, 1955) and dry weight of algal material (Castenholz, 1960, Grezenda and Brehmer, 1960) are long established methods.

Microscopic counts involve thorough scraping, mixing, and suspension of the algal cells. From this mixture an aliquot of cells is withdrawn for enumeration under a microscope. Dry weight is determined by drying and weighing the algal sample, then igniting the sample to burn off the algal materials, leaving inert inorganic materials that are again weighed. The difference between initial weight and weight after ignition is attributed to algae.

Any organic sediments, however, that settle on the artificial substrate along with the algae are processed also. Thus, if organic wastes are present appreciable errors may enter into this method.

3 During the past decade, chlorophyll analysis has become a popular method for estimating algal growth. Chlorophyll is extracted from the algae and is used as an index of the quantity of algae present. The advantages of chlorophyll analysis are rapidity, simplicity, and vivid pictorial results.

The algae are scrubbed from the artificial substrate samples, filtered to remove excess water, then each sample is placed (with filter) in equal volumes of acetone or alcohol, which extracts the chlorophyll from the algal cells. A second filtering to remove the extracted algae and other detritus produces a non-turbid, colored chlorophyl extract. The chlorophyll extracts may be compared visually.

Because the chlorophyll extracts fade with time, colorimetry should be used for permanent records. Harvey (1934) describes an artificial color standard. Richards with Thompson (1952) describes a method of colorimetry that determines the quantities of various kinds of chlorophyll along with other plant and animal pigments. For routine records, simple colorimeters will suffice. At very high chlorophyll densities, interference with colorimetry occurs, which must be corrected through serial dilution of the sample or with a nomograph

VII FACTORS INVOLVED IN DATA INTERPRETATION

Two very important factors in data evaluation are a thorough knowledge of conditions under which the data were collected and a critical assessment of the reliability of the data's representation of the situation.

A Maximum-Minimum Values

The evaluation of physical and chemical data to determine their effects on aquatic organisms is primarily dependent on maximum and minimum observed values. The mean is useful only when the data are relatively uniform. The minimum or maximum values usually create acute conditions in the environment.

B Identification

Precise identification of organisms to species requires a specialist in limited taxonomic groups. Many immature aquatic forms have not been associated with the adult species. Therefore, one who is certain of the genus but not the species should utilize the generic name, not a potentially incorrect species name. The method of interpreting biological data on the basis of numbers of kinds and numbers of organisms will typically suffice.

C Lake and Stream Influence

Physical characteristics of a body of water also affect animal populations. Lakes or impounded bodies of water support different faunal associations from rivers. The number of kinds present in a lake may be less than that found in a stream because of a more uniform habitat. A lake is all pool, but a river is composed of both pools and riffles. The nonflowing water of a lake exhibits a more complete settling of particulate organic matter that naturally supports a higher population of detritus consumers. For these reasons, the bottom fauna of a lake or impoundment cannot be directly compared with that of a flowing stream.

D Extrapolation

How can bottom-dwelling macrofauna data be extrapolated to other environmental components? It must be borne in mind that a component of the total environment is being sampled. If the sampled component exhibits changes, then so must the other interdependent components of the environment. For example, a clean stream with a wide variety of desirable bottom organisms would be expected to have a wide variety of desirable bottom fishes; when pollution reduces the number of bottom organisms, a comparable reduction would be expected in the number of fishes. Moreover, it would be logical to conclude that any factor that eliminates all bottom organisms would eliminate most other aquatic forms of life.

VIII IMPORTANT ASSOCIATED ANALYSES

A The Chemical Environment

1 Dissolved oxygen

2 Nutrients

3 Toxic materials

4 Acidity and alkalinity

5 Etc

B The Physical Environment

1 Suspended solids

2 Temperature

3 Light penetration

4 Sediment composition

5 Etc

IX AREAS IN WHICH BENTHIC STUDIES CAN BEST BE APPLIED

A Damage Assessment

If a stream is suffering from pollutants, the biota will so indicate A biologist can determine damages by looking at the "critter" assemblage in a matter of hours Usually, if damages are not found, it will not be necessary to alert the remainder of the agency's staff, pack all the equipment, pay travel and per diem, and then wait five days before enough data can be assembled to begin evaluation.

B By determining what damages have been done, the potential cause "list" can be reduced to a few items for emphasis and the entire "wonderful worlds" of science and engineering need not be practiced with the result that much data are discarded later because they were not applicable to the problem being investigated

C Good benthic data coupled with chemical, physical and engineering materials can be used to predict the direction of future changes and to estimate the amount of pollutants that need to be removed from the waterways.

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CASE PREPARATION AND COURTROOM PROCEDURE

I TYPES OF PROCEEDINGS IN WHICH WATER QUALITY EVIDENCE MAY BE USED

A Administrative Proceedings

1 Rule making

a Setting up of regulations having general application, e.g., stream classifications and implementation plan target dates

b Factors of safety and absolute prohibitions may be appropriate

2 Adjudications

a Determinations by agency having expertise with respect to particular discharge or discharger, e.g., approval of plans and specs and time schedule of a particular discharger

B Court Actions

1 Civil in behalf of state or federal government

a Actions to compel action or suspension of action - nuisance, health hazard, etc., --including court action following federal conference --hearing procedure

b Violations of Water Quality Standards

c Violations of Effluent Standards or discharge permits

d Tort or contract actions relating to design and/or operation of treatment facilities

2 Criminal (dependent on content of applicable statutes)

a Discharge of specific materials

b Discharges from specific industries

c Littering

d Discharges harmful to fish and/or crustaceans

e Discharges harmful to specific types of receiving waters

f Discharges of poisons

NOTE--In some of these situations doing the act may constitute the violation, in others proof of intent or knowledge of effects may also have to be proved.

3 Private actions for damages or to compel action

a Alleged harm to plaintiff, e.g., pollution of stream killing animals

C Procedural Matters

1 See Attached sheet "Administrative and Court Proceedings" on Burden of proof, fact finding, and methods of presentation of evidence.

D Classes of Evidence - General Rules

1 Facts - direct

a The material was floating from the outfall.

2 Derived values - expert testimony - test results and/or opinion as to effects

a The D.O. was zero, the waterway was polluted, the plant can be built in 6 months.

3 Hearsay

a Joe told me

4 Relevancy

5 Admissibility vs. weight

a Even if admissible, the weight to be given is up to fact finder--credibility.

E Admissibility of Results of Sampling and Testing (Numbers)

1 Sampling

a Chain of custody

b Tags, etc.

c Containers

d Place and time

e Retention of samples (Proving that the sample represents what is at issue in the action (relevancy), that there has been no opportunity for tampering, and availability of portions for analysis by other side (non-transitory criteria)).

2 Analysis

a Who performed (Can identity of each participant be shown?)

b Admission through supervisor custodian

c Scientific acceptance of method. Is there a particular method required to be used by the agency?

d Propriety of conduct

e Retention of bench cards and other indicia of results. (Your attorney can make arrangements to substitute copies for originals).

3 Tests

a Comparison with actual conditions

b Mathematical models - how can a computer be cross-examined?

F Admissibility of Expert Opinion on Causes and Effects

1 Who has special knowledge - and of what particular areas?

2 Indicators

3 Significance of numerical determinations or observations

4 Consistency with own prior publications and testimony

5 Have underlying facts been or need to be proved--first hand information of this and/or comparable situations.

6 Use of treatises

G Conduct on the Witness Stand

1 General

a On direct - know what counsel will ask and let him know generally what you will answer, but don't make it sound rehearsed.

b Use layman's language to extent possible.

c Listen to question and answer it to best of your ability.

d Speak so that court reporter, judge, jury, and counsel can hear you.

e Speak in language that will be understood, don't talk down.

f Answer only what you are asked --don't volunteer, however, answer with precision.

g There is nothing wrong with asking to have a question repeated or rephrased.

h There is nothing wrong with saying that you consulted with your attorney before you testified, but beware of the question "Did Mr. X tell you what to say?"

i There is nothing wrong with thinking out your answer before responding.

j You are not expected to know all the answers--if you do not know, admit it.

k Don't attempt to answer questions outside your area of personal knowledge (hearsay) or beyond your expertise. (Your may be an expert on conducting laboratory tests, but not on epidimeological inferences from results).

l Don't try to answer before the judge rules on objection.

m Show that you are an impartial dispenser of information and/or opinion, not a protagonist.

n Don't be afraid to admit what may appear to be damaging.

2 If you are testifying as an expert

a Establish qualifications -- give information relevant to your area of expertise -- educational (including this course?), work, publications, number of times you have testified previously.

b Differentiate between physical facts (measurements and observations) and opinion (derived values).

c Be prepared to discuss theory (including assumptions) instruments used, techniques(including choice of a particular technique), physical limitations and errors, interferences

d If experiments were conducted, be able to justify both as to theory and relevancy to this litigation.

e If you're being paid to testify, admit it.

3 Scientific personnel as advisers to counsel

a Review and refamiliarize self with materials before you discuss with your attorney.

b Be in a position to present all facts known to you simply and concisely Who, What, When, Where, and Why, How.

c Don't overlook facts and/or test results because you don't think they're important. Let attorney decide what he needs.

d Use of standard report forms

e Ability to recommend additional witnesses with needed specialized knowledge

f Ability to aid in cross-examination of other side's experts and reconcile opinions and/or results

g Be candid - sometimes better not to start a lawsuit or accept a settlement than lose in the end.

H Non-Verbal Presentation of Evidence

1 Exhibits - including photographs

2 Summaries

3 Business and/or government records

a Prepared contemporaneously and in usual course of activities

4 Pre-prepared direct examination

a Usually limited to actions before ICC, FPC, and other federal agencies.

I Criminal Procedure

1 Privilege Against Self Incrimination (available only to persons)

a Warning and suspects

b Effect of duty to report spills

c Effect of duty to obtain license or permit and/or furnish operating reports

d Immunity from prosecution

2 Double Jeopardy

3 Unreasonable search and seizure

a Available to persons and corporations

4 Procedures and need for arrest and search warrants -- possible cause

This outline was prepared by David I. Shedroff, Enforcement Analyst, Office of Enforcement and General Counsel, Cincinnati Field Investigations Center, 5555 Ridge Avenue, Cincinnati, OH 45268.

Administrative & Court Proceedings, and Excerpts from Revised Draft of Proposed Rules of Evidence for the United States Courts can be found on the following pages.

ADMINISTRATIVE & COURT PROCEEDINGS

Court or Agency	Fact Finder	Burden of Proof	Comments
State Pollution Control Agency	Agency	As per statute - usually weight of evidence.	Hearing may be conducted by hearing examiner, agency member, or full agency. Appeal may be on facts and law or law alone, depending on statute.
Rule making-adjudication			
Federal Water Pollution Control Act			
Conference	Head of agency		Reports acceptable.
Hearing	Hearing Board		Specific testimony.
Court	Judge		Uses prior material, and may take additional testimony.
Court			
Civil Case -- - for money only	Judge or jury	Weight of evidence	
- injunction			
preliminary or temporary	Judge	Must show immediate harm or danger.	Must also show likelihood of success at final hearing - bond required for non-government plaintiff.
permanent	Judge	Usually clear and convincing.	"Balance Equities"
- administrative appeal	Judge - whether "arbitrary and capricious" or substantial evidence		Sometimes have complete new trial.
Criminal case includes penalties	Jury unless waived.	Beyond reasonable doubt.	Proof of intent may be required.

Excerpts from Revised Draft of Proposed

RULES OF EVIDENCE FOR THE UNITED STATES COURTS

GENERAL PROCEDURES

Rule 102.

PURPOSE AND CONSTRUCTION

These rules shall be construed to secure fairness in administration, elimination of unjustifiable expense and delay, and promotion of growth and development of the law of evidence to the end that the truth may be ascertained and proceedings justly determined.

Rule 101.

PRELIMINARY QUESTIONS

(a) Questions of Admissibility Generally. Preliminary questions concerning the qualification of a person to be a witness, the existence of a privilege, or the admissibility of evidence shall be determined by the judge, subject to the provisions of subdivision (b). In making his determination he is not bound by the rules of evidence except those with respect to privileges.

(b) Relevancy Conditioned on Fact. When the relevancy of evidence depends upon the fulfillment of a condition of fact, the judge shall admit it upon, or subject to, the introduction of evidence sufficient to support a finding of the fulfillment of the condition.

Rule 615.

EXCLUSION OF WITNESSES

At the request of a party the judge shall order witnesses excluded so that they cannot hear the testimony of other witnesses, and he may make the order of his own motion. This rule does not authorize exclusion of (1) a party who is a natural person, or (2) an officer or employee of a party which is not a natural person designated as its representative by its attorney, or (3) a person whose presence is shown by a party to be essential to the presentation of his cause.

Rule 611.

MODE AND ORDER OF INTERROGATION AND PRESENTATION

(a) Control by Judge. The judge may exercise reasonable control over the mode and order of interrogating witnesses and presenting evidence so as to (1) make the interrogation and presentation effective for the ascertainment of the truth, (2) avoid needless consumption of time, and (3) protect witnesses from harassment or undue embarrassment.

(b) Scope of Cross-Examination. A witness may be cross-examined on any matter relevant to any issue in the case, including credibility. In the interests of justice, the judge may limit cross-examination with respect to matters not testified to on direct examination.

Rule 613.

PRIOR STATEMENTS OF WITNESSES

(a) Examining Witness Concerning Prior Statement. In examining a witness concerning a prior statement made by him, whether written or not, the statement need not be shown or its contents disclosed to him at that time, but on request the same shall be shown or disclosed to opposing counsel.

JUDICIAL NOTICE

Rule 201.

JUDICIAL NOTICE OF ADJUDICATIVE FACTS

(b) Kinds of Facts. A judicially noticed fact must be one not subject to reasonable dispute in that it is either (1) generally known within the territorial jurisdiction of the trial court or (2) capable of accurate and ready determination by resort to sources whose accuracy cannot reasonably be questioned.

(g) Instructing Jury. The judge shall instruct the jury to accept as established any facts judicially noticed.

RELEVANCE

Rule 401.

DEFINITION OF "RELEVANT EVIDENCE"

"Relevant evidence" means evidence having any tendency to make the existence of any fact that is of consequence to the determination of the action more probable or less probable than it would be without the evidence.

Rule 402.

RELEVANT EVIDENCE GENERALLY ADMISSIBLE,
IRRELEVANT EVIDENCE INADMISSIBLE

All relevant evidence is admissible, except as otherwise provided by these rules, by other rules adopted by the Supreme Court, by Act of Congress, or by the Constitution of the United States. Evidence which is not relevant is not admissible.

COMPETENCY OF WITNESSES

Rule 601.

GENERAL RULE OF COMPETENCY

Every person is competent to be a witness except as otherwise provided in these rules.

Rule 602.

LACK OF PERSONAL KNOWLEDGE

A witness may not testify to a matter unless evidence is introduced sufficient to support a finding that he has personal knowledge of the matter. Evidence to prove personal knowledge may, but need not, consist of the testimony of the witness himself. This rule is subject to the provisions of Rule 703, relating to opinion testimony by expert witnesses.

EXPERT TESTIMONY

Rule 702.

TESTIMONY BY EXPERTS

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.

Rule 703.

BASES OF OPINION TESTIMONY BY EXPERTS

The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence.

Rule 705.

DISCLOSURE OF FACTS OR DATA UNDERLYING EXPERT OPINION

The expert may testify in terms of opinion or inference and give his reasons therefore without prior disclosure of the underlying facts or data, unless the judge requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross-examination.

Rule 706.

COURT APPOINTED EXPERTS

(a) Appointment. The judge may on his own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The judge may appoint any expert witnesses agreed upon by the parties, and may appoint witnesses of his own selection. An expert witness shall not be appointed by the judge unless he consents to act. A witness so appointed shall be informed of his duties by the judge in writing, a copy of which shall be filed with the clerk, or at a conference in which the parties shall have opportunity to participate. A witness so appointed shall advise the parties of his findings, if any, his deposition may be taken by any party, and he may be called to testify by the judge or any party. He shall be subject to cross-examination by each party, including a party calling him as a witness.

HEARSAY

Rule 801.

DEFINITIONS

The following definitions apply under this Article:

(a) Statement. A "statement" is (1) an oral or written assertion or (2) nonverbal conduct of a person, if it is intended by him as an assertion.

(b) Declarant. A "declarant" is a person who makes a statement.

(c) Hearsay. "Hearsay" is a statement, other than one made by the declarant while testifying at the trial or hearing, offered in evidence to prove the truth of the matter asserted.

Rule 802.

HEARSAY RULE

Hearsay is not admissible except as provided by these rules or by other rules adopted by the Supreme Court or by Act of Congress.

Rule 803.

HEARSAY EXCEPTIONS: AVAILABILITY OF DECLARANT IMMATERIAL

The following are not excluded by the hearsay rule, even though the declarant is available as a witness

(5) Recorded Recollection. A memorandum or record concerning a matter about which a witness once had knowledge but now has insufficient recollection to enable him to testify fully and accurately, shown to have been made when the matter was fresh in his memory and to reflect that knowledge correctly. If admitted, the memorandum or record may be read into evidence but may not itself be received as an exhibit unless offered by an adverse party.

(6) Records of Regularly Conducted Activity. A memorandum, report, record, or data compilation, in any form, of acts, events, conditions, opinions, or diagnoses, made at or near the time by, or from information transmitted by, a person with knowledge, all in the course of a regularly conducted activity, as shown by the testimony of the custodian or other qualified witness, unless the sources of information or other circumstances indicate lack of trustworthiness.

(18) Learned Treatises. To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits.

IDENTIFICATION OF PERSONS AND SAMPLES

Rule 901.

REQUIREMENT OF AUTHENTICATION OR IDENTIFICATION

(a) General Provision The requirement of authentication or identification as a condition precedent to admissibility is satisfied by evidence sufficient to support a finding that the matter in question is what its proponent claims.

(b) Illustrations. By way of illustration only, and not by way of limitation, the following are examples of authentication or identification conforming with the requirements of this rule

(1) Testimony of Witness with Knowledge. Testimony that a matter is what it is claimed to be.

(3) Comparison by Trier or Expert Witness. Comparison by the trier of fact or by expert witnesses with specimens which have been authenticated

(9) Process or System. Evidence describing a process or system used to produce a result and showing that the process or system produces accurate result.

ADMISSIBILITY AND PROOF OF SPECIAL MATTERS

Rule 406.

HABIT, ROUTINE PRACTICE

(a) Admissibility. Evidence of the habit of a person or of the routine practice of an organization, whether corroborated or not and regardless of the presence of eye-witnesses, is relevant to prove that the conduct of the person or organization on a particular occasion was in conformity with the habit or routine practice.

(b) Method of Proof. Habit or routine practice may be proved by testimony in the form of an opinion or by specific instances of conduct sufficient in number to warrant a finding that the habit existed or that the practice was routine.

Rule 612

WRITING USED TO REFRESH MEMORY

If a witness uses a writing to refresh his memory, either before or while testifying, an adverse party is entitled to have it produced at the hearing, to inspect it, to cross-examine the witness thereon, and to introduce in evidence those portions which relate to the testimony of the witness.

Rule 1006.

SUMMARIES

The contents of voluminous writings, recordings, or photographs which cannot conveniently be examined in court may be presented in the form of a chart, summary, or calculation. The originals, or duplicates, shall be made available for examination or copying, or both, by other parties at a reasonable time and place The judge may order that they be produced in court.

BACTERIA AND PROTOZOA AS TOXICOLOGICAL INDICATORS

I INTRODUCTION

The WINOGRADSKY COLUMN is an excellent simple classroom experiment as well as a miniature ecosystem which yields a variety of photosynthetic and other protista especially the bacterial forms important in photosynthesis research. These photosynthetic bacteria, as pointed out by Dr. Hutner, are ubiquitous in wet soils and natural waters but ordinarily escape notice.

II PREPARING THE COLUMN

The materials needed are simple laboratory items.

A The inoculum (a black sludge) may be easily obtained from a local sewage treatment plant or the bottom of a pond or lake. Because the USPHS document, containing Dr. Hutner's paper, is out of print it is reproduced here.

B Directions for preparing the column and other useful information are given in that paper.

C Dr. Hutner's bibliography should be sufficient for those who wish more information.

III ECOSYSTEM DEVELOPMENT

A Factors such as the substrate used, the inoculum, the overlying supernatant water, and laboratory conditions as temperature and light, will all influence the particular type of biota forming successive layers or zones. The accompanying figure is therefore generalized and is not intended to be absolute.

B Some representative forms are listed for general information. The numbers correspond to those on the figure.

1 Inorganic substrate on toweling.

2 Green photosynthetic bacteria Microchloris, Chlorobium, and Chlorochromatum, methane bacteria, and SO_4 reducers.

3 Photosynthetic purple sulfur bacteria. Thiopedia and Thiosarcina.

4 Filamentous sulfur bacteria, Beggiatoa.

5 Non-sulfur photosynthetic bacteria, Rhodopseudomonas.

6 Blue-green algae, Schizothrix and Oscillatoria.

7 Diatoms, Nitzschia.

8 Coccoid green algae, Ankistrodesmus, and flagellate greens, Chlamydomonas, similar to a stabilization pond flora.

9 Filamentous green algae, Ulothrix.

C Besides the photosynthetic bacteria and other protista there will be a variety of protozoa found in the aerobic levels (app. 6 through 9). Many of these protozoans are typical fauna of activated sludge.

D The possibilities are endless for further experimentation. These ecosystems are also convenient and inexpensive sources for protozoa and other protista for class and laboratory instruction.

IV MICRO AQUARIA

A Fenchel describes a micro aquarium (1.5 X 5 cm) which may be observed under a compound microscope. (Figure 2)

B The development of communities of organisms is quite similar to the Winogradsky Column. (Figure 3)

C The basic media consists of one liter of natural water 10 g $CaSO_4$, 1 g glucose, 1 g of peptone, autoclaved and stored at 5° C.

D Before use agar is boiled with the media. While hot, the media is introduced into one end of the "micro aquarium" with a pipet. After the agar congeals, natural water samples are added. During incubation and when not being observed, the micro aquaria are kept in a moist chamber.

E Although Fenchel used a seawater medium and inoculum, freshwater sources would be equally useful.

F In these microaquaria simple ecosystems develop when they are kept in complete darkness. Complex photosynthetic communities develop when they are illuminated. A natural ecosystem is figured by Fenchel (Figure 4), and a related food web is shown in Figure 5 (both from Fenchel).

G Microaquaria Using Plastic Petri Dishes

Sessile ciliates have been successfully collected, cultured, and used for bioassay using the same petri dish (membrane filter type, with tight fitting lids).

H Microaquaria using silicone cement rings which allow diffusion of gases through the silicone cultures will thereby remain active indefinitely.

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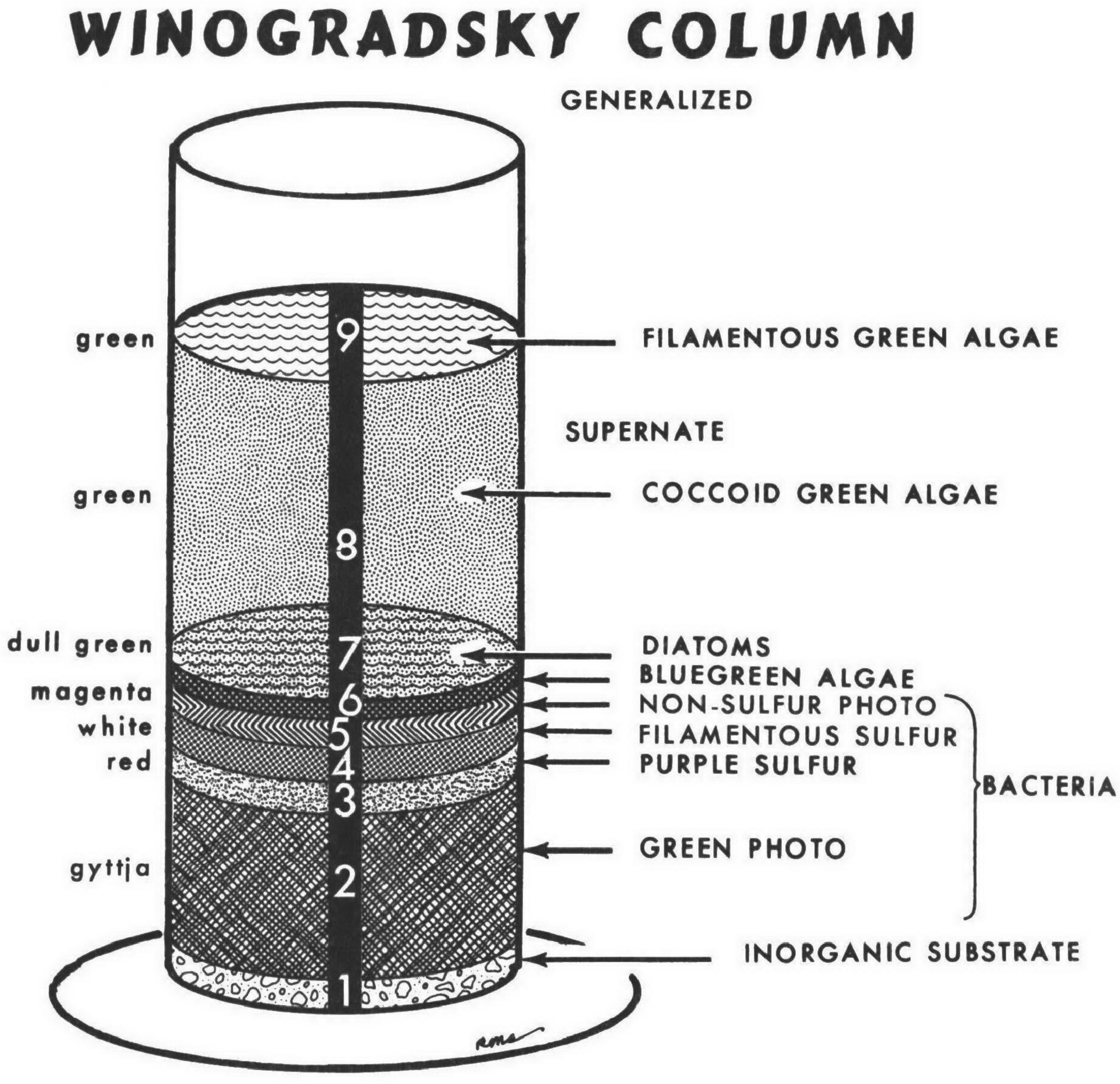
WINOGRADSKY COLUMN
GENERALIZED
green
green
dull green
magenta
white
red
gyttja
9
8
7
6
5
4
3
2
1
FILAMENTOUS GREEN ALGAE
SUPERNATE
COCCOID GREEN ALGAE
DIATOMS
BLUEGREEN ALGAE
NON-SULFUR PHOTO
FILAMENTOUS SULFUR
PURPLE SULFUR
GREEN PHOTO
BACTERIA
INORGANIC SUBSTRATE

FIGURE 1

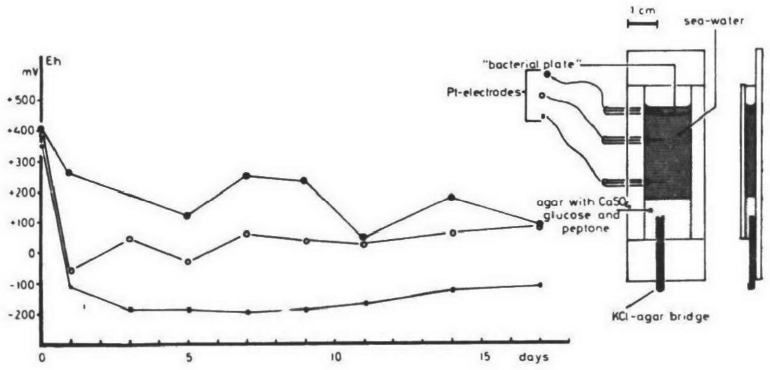

FIGURE 2

A micro aquarium fitted with electrodes and the redox conditions through 17 days.

FIGURE 3

Drawing made by tracing a micrograph of the bacterial plate in a micro aquarium (same experiment as shown on Figure 2.) Most conspicuous are the filaments of Beggiatoa and the ciliates Cyclidium citrullus, Euplotes elegans and Holosticha sp. Below the Oscillatoria filament (lower left) a Plagiopogon loricatus is seen. Bacteria (except Beggiatoa) are not shown.

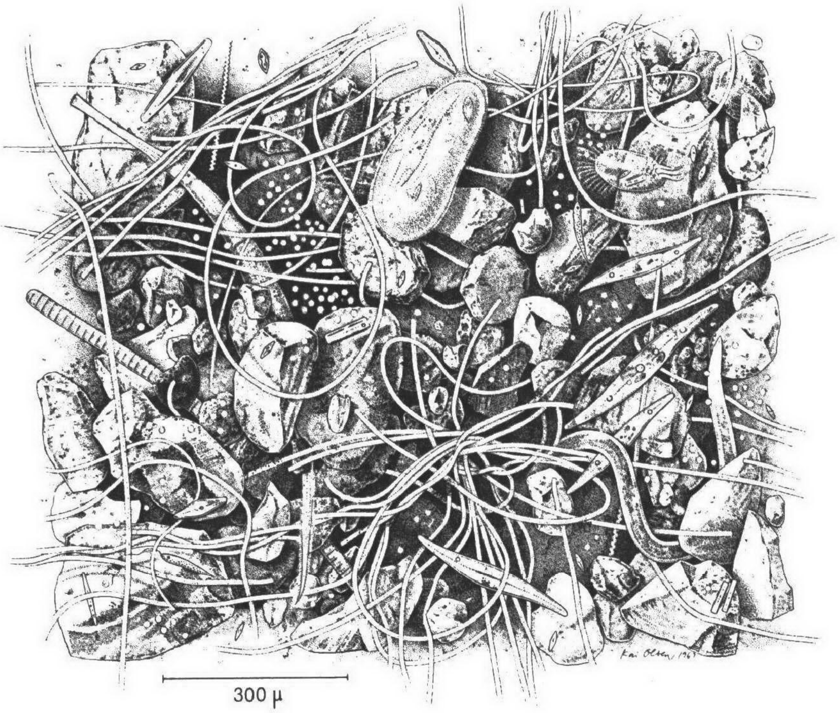

FIGURE 4

The microflora and fauna in the surface of the Beggiatoa patches. (Oscillatoria, Beggiatoa, Thiovolum, diatoms, euglenoids, nematode, Trachelorahis sp., Frontonia marina, Diophrys scutum, Trochiloides recta).

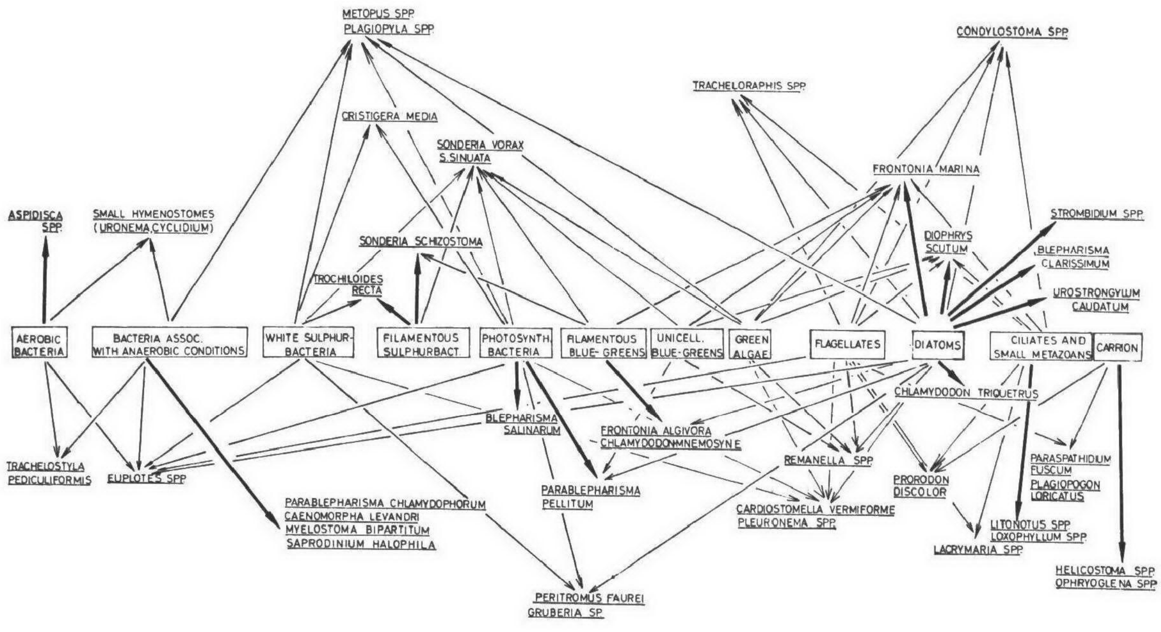

FIGURE 5

The food relationships of the most common ciliates in "estuarine" sediments and in sulphureta.

ENVIRONMENTAL REQUIREMENTS OF FRESH-WATER INVERTEBRATES

L. A. Chambers, *Chairman

Bacteria-Protozoa as Toxicological Indicators in Purifying Water

S. H. Hutner, Herman Baker, S. Aaronson and A. C. Zahalsky+

There is a cynical adage that all travelers become entomologists. But, now with DDT and detergents, travelers and stay-at-homes alike are becoming toxicologists. We have an immediate interest in pollution problems Our laboratory receives, like the East River and adjoining United Nations buildings, a generous sootfall from a nearby power plant. Also, we have seen a superb fishing ground, Jamaica Bay in New York City, become a sewer. (Jamaica Bay is, however, being restored to its pristine cleanliness--but not the U.N. area.) We take our theme nevertheless not from aesthetics but from statements by Berger (1961): (1) It is an expensive, time-consuming project"...to predict with confidence a new waste's probable impact on certain important down-stream water uses." And (2) "The toxicological phase of the study is perhaps its most perplexing aspect. The specialized services and cost necessary for determining the effect of repeated exposure to low con-centrations of the waste for long periods of time would inevitably place this job out of reach of most public agencies. Equally discouraging, perhaps, is the probability that the toxicological study may take as long as two years."

As described here, advances in microbiology offer hopes of lightening this burden. The first question is What kind of microcosm will serve for toxicological surveys, especially for predicting the poisoning of biological means of waste disposal? The second is: Can the protozoa of this microcosm predict toxicity to higher animals?

The food-chain pyramids of sewage plants and polluted waters have been adequately described (Hynes, 1960, Hawkes, 1960). A problem treated here is how to scale those microcosms to experimentally manipulable microcosms yielding dependable predictions for the behavior of sewage-plant microcosms.

THE WINOGRADSKY COLUMN AS SOURCE OF INOCULA FOR MINERALIZATIONS AND AS TOXICOLOGICAL INDICATOR SYSTEM

Total toxicity depends on intrinsic toxicity-persistence relationship. Techniques for testing the degradability of organic compounds--and so their persistence in soil and water--are still haphazard. The enrichment culture technique, in which one seeks microbes that use the compound in question as sole substrate--hence degrades it and even "mineralizes" it--was developed by the Dutch school of microbiologists. Enrichment cultures are used routinely by biochemists wishing to work out the microbial catabolic metabolic pathway for a compound of biochemical interest. Since the compounds dealt with by biochemists are of biological origin, micro-bial degradability can be assumed. Still, finding a microbe to degrade a rare biochemical is not always easy Dubos, in a classical hunt for a microbe able to live off the capsules of pneumococci, found the bacterium only after a long search which ended in a cranberry bog. Such difficulty in finding microbes that degrade rare biochemicals implies an even greater difficulty in finding microbes that degrade many products of the synthetic

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organic chemicals industry, since such compounds may embody biochemically rare or biochemically nonexistent linkages. Intimations of the importance of inoculum abound in the literature, e.g., Ross and Sheppard (1956) could not at first obtain phenol oxidizers from ordinary inocula (presumably soil and water), but manure and a trickling filter from a chemical plant proved abundant sources of active bacteria. One wonders how extensive a study underlies the statement quoted by Alexander (1961) that "soils treated with 2, 4, 5-T (trichlorophenoxy-acetic acid) still retain the pesticide long after all vestiges of toxicity due to equivalent quantities of 2, 4-D have disappeared."

What then is a reasonable inoculum for testing a compound's susceptibility to microbial attack? The size range is wide from the traditional crumb or gram of soil or mud to the scow-load of activated sludge contributed by New York City to inaugurate the Yonkers sewage-disposal plant. We suggest that to strike a practical mean in getting a profile of soil, mud, or sludge to be used as inoculum the uses of the Winogradsky column should be explored. Directions for Winogradsky column and bacteriological enrichments have been detailed (Hutner, 1962) and so only an outline is given here. The column is prepared by putting a paste of shredded paper, $CaCO_3$, and $CaSO_4$ at the bottom of a hydrometer jar, filling the jar with mud smelling H_2S, covering with a shallow layer of water, and illuminating from the side with an incandescent lamp. In 2 or 3 weeks sharp zones appear: a green-and-black anaerobic zone at the bottom (a mixture of green photosynthetic bacteria along with SO_4-reducers, methane producers, and the like), over that a red zone (predominantly photosynthetic bacteria), above this garnet or magenta spots or zone (predominantly non-sulfur photosynthetic bacteria), above this a layer rich in blue-green algae (the transition to the aerobic zones), above this aerobic bacteria along with green algae, diatoms, other algae, and an assortment of protozoa. This makes an excellent simple classroom experiment to demonstrate the different kinds of photosynthetic organisms, especially the bacterial forms that are important in photosynthesis research and that ordinarily escape notice yet are ubiquitous in wet soils and natural waters.

As pointed out by our colleague, Dr. L. Provasoli (1961), the heterotrophic capacities of algae are very imperfectly known. This is underscored by recent studies of the green flagellate Chlamydomonas mundana as a dominant in sewage lagoons in the Imperial Valley of California (Eppley and MaciasR, 1962), other than that it prefers acetate among the few substrates tried, its heterotrophic capacities are unknown. More unexpectedly, some strains of the photosynthetic bacterium Rhodopseudomonas palustris use benzoic acid anaerobically as the reductant in photosynthesis (Scher, Scher, and Hutner, 1962) narrowing the gap between the photosynthetic pseudomonads and the ubiquitous pseudomonads so often represented among bacteria attacking resistant substrates (e.g., hydrocarbons) as well as highly vulnerable substrates. For the widely studied, strongly heterotrophic photosynthetic flagellates Euglena gracilis and E. viridis, common in sewage, no specific enrichment procedure is known, meaning that their ecological niches are unknown but laboratory data provide hints.

The increasing use of oxidation ponds would in any case urge a greater use of scaled-down ecological systems in which development of none of the photosynthesizers present in the original inoculum was suppressed. Conceivably, some of the rare microbes attacking rare substrates--and such microbes are likely to represent a source of degraders of resistant non-biochemicals--are specialists in attacking products of photosynthetic organisms.

Traditionally, the inoculum for a Winogradsky column is a marine or brackish mud (as New Yorkers we would be partial to mud from flats of the Harlem River). Little is known about the effectiveness as inocula of freshwater muds or water-logged soils. It would be valuable to know how complete a column could develop from material from a trickling filter or an activated-sludge plant. A practical issue is: Might the poisoning of a sewage-oxidation system be paralleled by the poisoning of a Winogradsky column, where the poison was mixed with the inoculum for the column? Might the variously colored photosynthetic zones of the column and the aerobic population on top provide sensitive indicators for the performance of a sewage-oxidation system subjected to chemical wastes?

If a particular compound mixed with inoculation mud or sludge suppressed development of the full Winogradsky pattern, one might assume that the compound at the test concentration was poisonous and persistent. Biological destruction of such poisons, if at all possible, might demand a long hunt for suitable microorganisms, then buildup of the culture to a practical scale. This might best be done with illuminated shake or aerated cultures, with the inocula coming from a variety of environments. Optimism that microbes can be found capable of breaking almost all the linkages of organic chemistry is fostered by the study of antibiotics, which include a wealth of previously "unphysiological" linkages--azo compounds, oximes, N-oxides, aliphatic and aromatic nitro and halogen compounds, and strange heterocyclic ring systems. Some natural heterocycles, e.g., pulcherriminic acid and 2-n-nonyl-4-hydroxyquinoline, have a disquieting resemblance to the potent carcinogen 4-nitroquinoline N-oxide.

PROTOZOA AS TOXICOLOGICAL TOOLS

A difficult problem is one mentioned earlier. persistence joined with low-grade toxicity to higher animals. Recent developments in the use of protozoa as pharmacological tools show that protozoa can serve as sensitive detectors of metabolic lesions ("side actions"?) of a wide assortment of "safe" drugs. The list includes the "anticholesterol" triparanol (MER/29). Triparanol toxicity manifested itself with several protozoa, including Ochromonas danica (Aaronson et al., 1962) and Tetrahymena (Holz et al., 1962). Triparanol was not acting simply as an anticholesterol for its obvious toxicity to protozoa was annulled by fatty acids as well as by sterols. The connection between the protozoan results and the "side actions" of triparanol--baldness, impotence, and cataracts--are of course unclear, but protozoal toxicity might serve as an initial warning that it might not be as harmless as assumed from short-term experiments with higher animals.

The anticonvulsant primidone provides a clear indication of how protozoa can be used to pinpoint the location of a metabolic lesion. Primidone had been known to cause folic acid-responsive anemias. It is therefore easy to find that with joint use of a thymine-dependent Escherichia coli and the flagellate Crithidia fasciculata reversal of growth inhibition by folic acid and related compounds permitted the charting of interferences with the interconnected folic acid, biopterin, and DNA function (Baker et al., 1962), which amply accounted for the megaloblastic anemias. Lactobacillus casei, a bacterium much used in chemotherapeutic research, was unaffected by the drug.

In another instance, where the mode of action of the drug in higher animals was unknown, growth inhibition property of the anticancer compound 1-aminocyclopentane-1-carboxylic acid was reversed for Ochromonas danica by L-alanine and glycine, as was the inhibition property of 1-amino-3-methyl-cyclohexane-1-carboxylic acid by L-leucine (Aaronson and Bensky, 1962).

Growth inhibition of Euglena by the potent carcinogen 4-nitroquinoline N-oxide was annulled by a combination of tryptophan, tyrosine, nicotinic acid, phenylalanine, uracil (Zahalsky et al., 1962) and, in more recent experiments, the vitamin K relative phthiocol. These N-oxides are of interest because of recent work indicating that perhaps the main way in which the body converts such compounds as the amino hydrocarbons to the actual carcinogens may be by an initial hydroxylation of the nitrogen, e.g., work by Miller et al., (1961). Whether the peroxides in photo-chemical smogs of the Los Angeles type act on hydrocarbons to produce carcinogenic N-oxides is entirely unknown. Leighton (1961) lists an array of peroxy reactions produced by sunlight in polluted air.

Our aforementioned experience with primidone, a ketonic heterocycle, led us to test the sedative thalidomide. It was toxic for Ochromonas danica O. malhemensis and Tetrahymena pyriformis, this toxicity was annulled by nicotinic acid (or nicotinamide) or vitamin K (menadione) (Frank et al, 1963). We do not know whether a similar protection could have been conferred on human embryos or polyneuritis in the adult.

Many widely used herbicides of the dinitrophenol type are powerful poisons for higher animals. We do not know how sensitive protozoa would be for detecting their persistence. However, since somewhat similar thyro-active compounds can be sensitively detected by their exaggeration of the B_{12} requirement of Ochromonas malhamensis (Baker et al, 1961), this flagellate might be a useful test object for dinitrophenols and congeners.

The Paramecium (and perhaps too the Tetrahymena) test for polynuclear benzenoid carcinogens has remarkable sensitivity and specificity (Epstein and Burroughs, 1962, Hull, 1962). This test depends on the carcinogen-sensitized photodynamic destruction of paramecia by ultraviolet light. This test is approaching practicality for air, and there is no reason to suppose it cannot be applied to benzene extracts of foodstuffs and water.

CONCLUSIONS

We have suggested here new procedures for examining the intrinsic toxicity-persistence relationship, using as test organisms the protists represented conspicuously in a Winogradsky column. The new field of micro-toxicology is virtually undeveloped. The urgent need for detecting chronic, low-grade toxicities is evident from many sides. This is not the place for a detailed discussion of the medical implications of this area of research, but it should be emphasized that chronic toxicities and carcinogenesis are related. Conversely, Umezawa (1961) has remarked that most antitumor substances have chronic toxicities and that elaborate testing procedures for toxicity are required to fix the daily tolerable dose, apparently this problem is a central theme in medical as well as pollution research. Inhibition of growth of an array of protozoa is now in practical use as a means of detecting anticancer substances in antibiotic beers (Johnson et al, 1962).

Since the embryos appear to lack the detoxication mechanisms of the adult animal (Brodie, 1962), toxicity for protozoa (which presumably lack these detoxication mechanisms) should be compared with that for the embryo, not the adult, as emphasized by the thalidomide disaster.

There are further limitations on the use of microbes as detectors of toxicity. High-molecular toxins seem inert to microbes, and antihormones (with the exception of anti-thyroid compounds) are generally inert. The main usefulness of microbial indices of toxicity would appear, then, to be for detecting low-molecular poisons acting on cellular targets rather than on cell systems and organs. These are precisely the poisons likely to put out of business a pollution-control installation primarily dependent on microbial activity.

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