PLANKTON ANALYSIS

CLEAN WATER

TRAINING MANUAL

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION
U.S. DEPARTMENT OF THE INTERIOR

PLANKTON ANALYSIS

This course is offered for professional personnel in the fields of water pollution control, limnology, and also water supply. Primary emphasis is given to practice in the identification and enumeration of organisms which may be observed in the microscopic examination of water. Problems of significance and control are also considered.

U.S. DEPARTMENT OF THE INTERIOR
Federal Water Pollution Control Administration
TRAINING PROGRAM

May 1970

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Federal Water Pollution Control Administration.

Due to the limited production and 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 is for illustration only; such references do not imply product endorsement by the Federal Water Pollution Control Administration or the U.S. Department of the Interior.

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.

H. M. Freeman

H. M. Freeman
Chief, Direct Training Branch
Division of Manpower and Training
Federal Water Pollution Control
Administration

TRAINING PROGRAM

The Federal Water Pollution Control Administration of the U. S. Department of the Interior conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

Training is available at five installations of the Administration. These are: the National Training Center located at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio; the Robert S. Kerr Water Research Center, Ada, Oklahoma; the Southeast Water Laboratory, Athens, Georgia; the Pacific Northwest Water Laboratory, Corvallis, Oregon, and the Hudson-Delaware Basins Office, Edison, New Jersey.

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. These courses are being designed for nontechnical audiences, including administrators at the policy and decision-making levels, representatives of public action groups, and others not requiring the depth of detail of the more specialized courses.

Scientists, engineers, and recognized authorities from other FWPCA 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 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 Federal Water Pollution Control Administration, and the loan of instructional materials such as lesson plans and visual training aids, may be obtained through special arrangement. These training aids, including 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.

An annual Bulletin of Courses is prepared and distributed by the Water Pollution Control Training Program. 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 one of the training centers mentioned above.

U.S. DEPARTMENT OF THE INTERIOR

Federal Water Pollution Control Administration

MANPOWER AND TRAINING DIVISION

DIRECT TRAINING BRANCH

H. M. Freeman, Chief

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R. M. Sinclair, Aquatic Biologist
C. E Sponagle, Sanitary Engineer

HUDSON-DELAWARE BASINS OFFICE
Edison, New Jersey

F. P. Nixon, Deputy Regional Training Officer
R. B. Fagan, Aquatic Biologist

PLANKTON ANALYSIS (141)

2 weeks

CINCINNATI, OHIO

May 11–22, 1970
October 5–16, 1970

This course is offered for professional personnel concerned with the evaluation of natural and polluted waters by means of plankton examination. Limited attention is also devoted to the examination and interpretation of the fauna of activated sludge and waste stabilization ponds.

Instruction enables the student to carry out basic laboratory procedures in the identification and counting of both phytoplankton and zooplankton. He will be capable of applying taxonomic procedures to plankton and recognize the major types he is likely to encounter. He will be able to calibrate a microscope and to carry counting and group identification to the point of obtaining results which are qualitatively and quantitatively reliable.

Attention is given to the significance of various types of counts. Forms frequently found in water and wastewater treatment plants and polluted environments are emphasized. Techniques for plankton control are presented. Time is provided for discussion of local problems, both in class and with specialists at the training facility.

Representative course topics usually include

- Water quality problems of biological origin
- Identification of planktonic animals and plants
  - (a series of lectures and laboratories comprising approximately half of the course)
- Microscope calibration
- Plankton analysis
  - Sampling and preparation
  - Techniques of counting
  - Enumeration, methods selection
- Plant operation problems
  - Plankton in stabilization ponds
  - Activated sludge fauna
  - Toxic algae
  - Other biological treatment problems
- Plankton control
  - Plant control
  - Control in surface waters

Although microscopes are available for class use, more effective training results when it is given on the same instrument that will be used in the home laboratory. The microscope should have magnifications up to approximately 400X, oil immersion is optional. The student consequently is urged to hand-carry his own microscope to the course.

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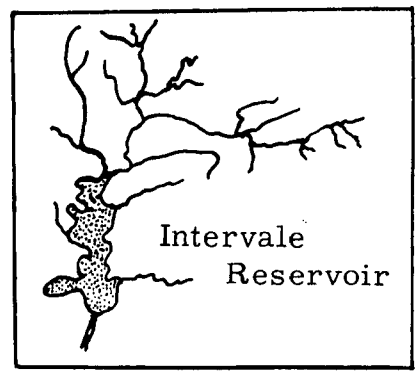


CHAPTER I

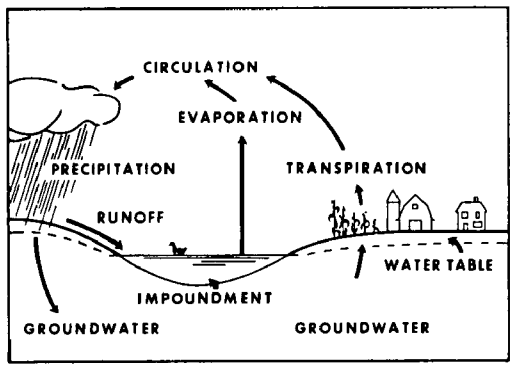
INTRODUCTION

Water Resources and Needs 1
Limnology and Ecology of Plankton 2
Optics and the Microscope 3
The Aquatic Environment 4

WATER RESOURCES AND NEEDS

I WATER RESOURCES

A The source of all freshwater is the hydrologic cycle, shown in Figure 1.



THE HYDROLOGIC CYCLE

Figure 1

1 Precipitation of water as rain, snow, hail, sleet or dew.

2 Percolation of water through soil to an aquifer to form groundwater.

3 Runoff of water which forms lakes, streams and rivers.

4 Evaporation of surface water or transpiration of water from green plants to the atmosphere.

5 Atmospheric recirculation of the water vapor.

B The world's supply of water is contained within the hydrologic cycle as:

1 Oceanic water

2 Water vapor in the atmosphere

3 Ice and snow in glaciers and snowpack

4 Runoff water in lakes and streams

5 Groundwater

C Withdrawals for use are mostly from those waters in the runoff and groundwater phases, although some oceanic waters are being utilized.

D Precipitation--which serves to recharge groundwaters and surface supplies--is at a relatively fixed annual rate.

1 Average precipitation in the U.S. is 30 inches per year or 3,900 billion gallons per day.

2 Evapo-transpiration losses total approximately 21 inches per year or approximately 2,740 billion gallons per day.

3 The available water totals approximately 9 inches per year or 1,160 billion gallons per day.

II THE DISTRIBUTION OF U.S. WATER RESOURCES

Although the water supply in the hydrologic cycle is fixed in amount, it is not distributed evenly. A wide disparity of water distribution exists both in time and space. Distribution of the annual average precipitation is shown in Figure 2.

A Distribution of Precipitation

1 Dependent upon:

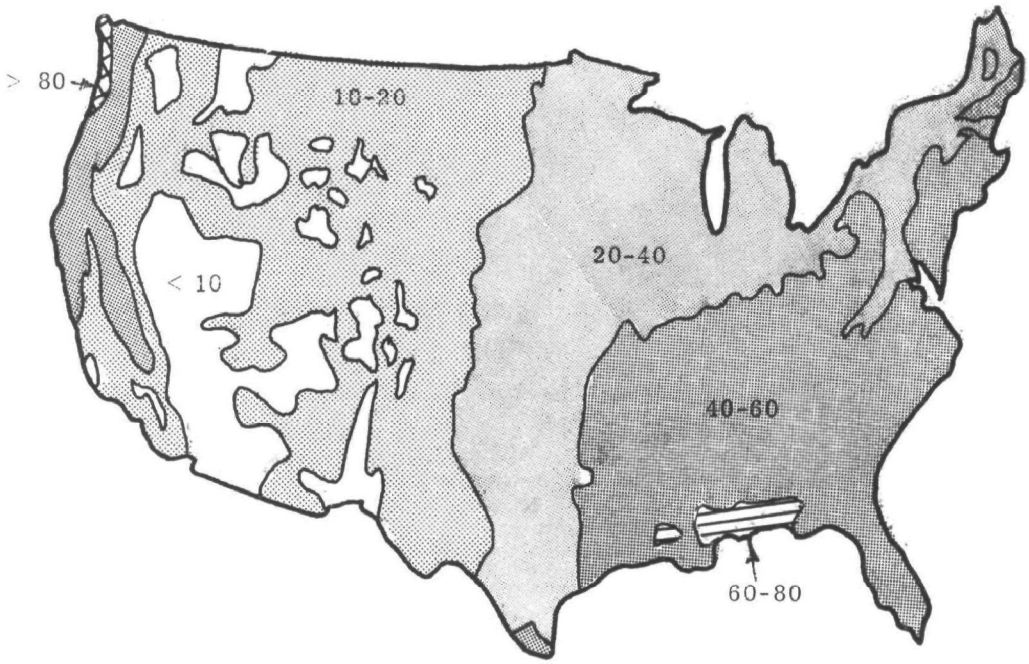
a Atmospheric conditions such as temperature and winds

b The geography of the region

c The general climate of the area

2 U.S. areas of high annual precipitation

a The Pacific slope varies from 10 inches to greater than 100 inches annually.



Distribution of Precipitation
(Average Annual)
Inches

Figure 2

b The gulf states precipitation varies from 20 to 60 inches annually.

c Precipitation in the midwest and Great Lakes area ranges from 25 to 50 inches per year.

d Precipitation along the Atlantic Coast averages between 35 to 50 inches per year.

3 Areas of low annual precipitation

a The Rocky Mountain area precipitation ranges between 10 and 20 inches per year.

b Much of the southwest has less than 10 inches of precipitation annually.

4 Distribution of precipitation with time

a The rainy or wet season varies from summer to winter, or in some areas there is relatively little change throughout the year.

b Local storms of high intensity may reach as much as 30 inches in 24 hours.

B Distribution of Runoff

1 Dependent upon:

a Precipitation in the region

b Infiltration - which is controlled by the geologic formations and the time lapse between rains.

c Season of the year controls evaporation, and snow melt.

d Topography controls the time available to percolate through the soil.

e Vegetation type and density affects interception and evapotranspiration.

2 Areas of high annual runoff

a Sections of the Pacific slope have greater than 80 inches annually.

b The eastern 1/3 of the U.S. averages greater than 20 inches of runoff annually.

3 Much of the western U.S. has less than 1 inch of runoff annually.

a Southwest

b Rocky Mountain states

c Rocky Mountain plateau

4 Time distribution of runoff

a Overflow--runoff during and immediately following precipitation.

b Base flow--sustained or fair weather runoff composed of delayed subsurface and groundwater runoff. See Figure 3 for runoff cycle.

C Distribution of Groundwater

1 Groundwater volume is affected by the same factors as runoff.

2 Geologic formations and soils control percolation and storage of groundwater.

3 Topography controls time available for percolation.

4 Evapo-transpiration varies with the season, as does precipitation and ground saturation.

III WATER USE

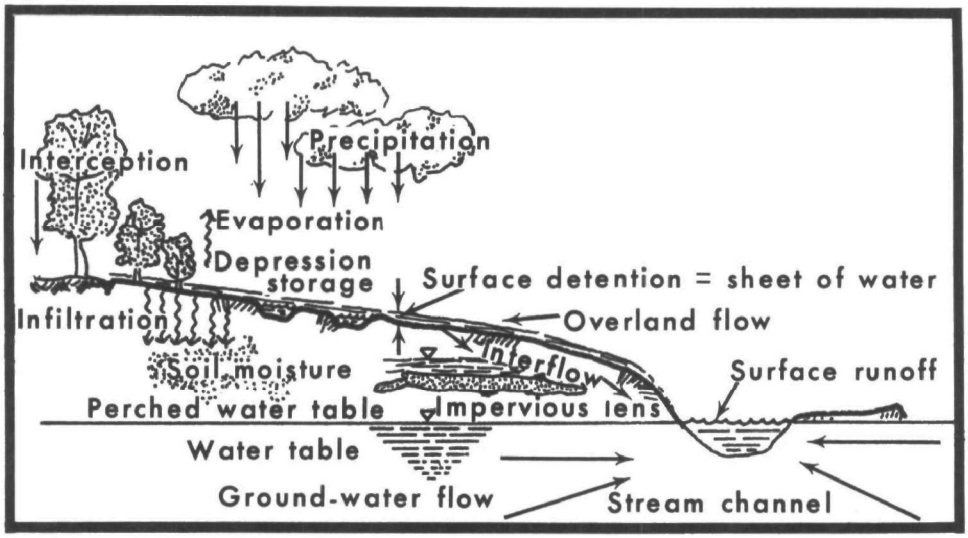
A Present Water Use in the U.S.

1 Water available for use

a Nine inches or 1,160 billion gallons per day are not lost through evapotranspiration, and is therefore theoretically available.

b Water use in the U.S. at the present time is approximately 390 billion gallons per day or 3 inches of our total supply.

c Twenty-one inches are lost through evapo-transpiration.



THE RUNOFF CYCLE
(Davis & DeWiest)
Figure 3

2 The way in which water is used

Water uses can be grouped into two classes. Those uses which are in situ such as recreation, fishing, and wildlife and those uses requiring withdrawal from the stream. These withdrawals are:

a Agricultural uses take 46% of our supply or 180 billion gallons/day; only 40% of this water is returned to the streams.

b Industrial uses take another 46% of our supply. 2% of the water used by industry is consumed.

c Municipal uses total approximately 25 billion gallons daily or 8% of the total.

3 Source of water used in U.S.

a National averages show 80% or 312 billion gallons per day to be from surface sources, while 20% is taken from the ground.

b The ratio of surface water to groundwater varies and is dependent on the quantity and quality available in each locality, as well as the cost.

4 Seasonal uses of water

a Irrigation waters are used during the growing season only.

b Some water using industries such as the canning industry are seasonal.

c The majority of industries needs water throughout the year.

d Municipal use is higher in the summer.

B Demand for water is increasing

1 The predicted demand of water in 1980 is approximately 600 billion gallons of water per day, or 220,000 billion per year.

2 This is mainly due to expansion of industry and irrigated agriculture.

3 Much of the demand for water will be in areas such as the southwest, that are already short on water.

C Methods for the Development of U.S. Water Resources for Future Needs

1 Utilization of our present sources of water, surface and groundwater, must be increased. This would mean increased storage, both on the surface and in underground reservoirs.

2 Desalinization of ocean waters and brackish waters holds some promise for regions where transportation will not be expensive.

3 Reduction of evapo-transpiration losses will greatly increase our total available supply.

4 Weather modification methods could possibly give us precipitation in the right place at the right time.

5 Greater reuse of our present supply is both through multiple use and better waste treatment methods.

IV SUMMARY

The total amount of water available appears to be fixed. In view of the increasing demands and the currently inefficient utilization of the supply, the demand may very shortly exceed the supply. Better management of the resource and more engineering research are urgently needed.

Table 1. AVAILABILITY OF GROUND WATER

Areas		Water Use (excluding water power) Use in mgd and Percent of total from Ground Water Sources	
		Total mgd	Ground water (%)
A	Atlantic and Gulf Coastal Plain area	32,000	25
B	Southern Great Plains area	21,000	45
C	Appalachian Mountain and Piedmont area	8,000	50
D	Rocky Mountains, northern Great Plains, and northern Pacific Coast area	28,000	12
E	Unglaciated central plateaus and lowlands	26,000	10
F-1	Basin and range	41,000	42
F-2	Columbia Plateau	24,000	7
G	Glaciated area of the East and Midwest	57,000	10
	U.S. Total (rounded)	240,000	20

ACKNOWLEDGEMENT:

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This outline was prepared by Edward D. Schroeder, Former Engineer, FWPCA Training Activities, SEC and revised by L. J. Nielson, Chief Technical Training, Pacific Northwest Water Laboratory, Corvallis, Oregon.

LIMNOLOGY AND ECOLOGY OF PLANKTON

I INTRODUCTION

A Most Interference Organisms are Small.

B Small Organisms generally have Short Life Histories.

C Populations of Organisms with Short Life Histories may Fluctuate Rapidly in Response to Key Environmental Changes.

D Small Organisms are Relatively at the Mercy of the Elements

E The Following Discussion will Analyze the Nature of These Elements with Reference to the Response of Important Organisms.

II PHYSICAL FACTORS OF THE ENVIRONMENT

A Light is a Fundamental Source of Energy for Life and Heat.

1 Insolation is affected by geographical location and meteorological factors.

2 Light penetration in water is affected by angle of incidence (geographical), turbidity, and color. 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.

3 Turbidity may originate within or outside of a lake.

a That which comes in from outside (allochthonous) is predominately inert solids (tripton).

b That of internal origin (autochthonous) tends to be biological in nature.

B Heat and Temperature Phenomena are Important in Aquatic Ecology.

1 The total quantity of heat available to a body of water per year can be calculated and is known as the heat budget.

2 Heat is derived directly from insolation; also by transfer from air, internal friction, and other sources.

C Density Phenomena

1 Density and viscosity affect the floatation and locomotion of microorganisms.

a Pure fresh water achieves its maximum density at $4^{o}C$ and its maximum viscosity at $0^{o}C$.

b The rate of change of density increases with the temperature.

2 Density stratification affects aquatic life and water uses.

a In summer, a mass of warm surface water, the epilimnion, is usually present and separated from a cool deeper mass, the hypolimnion, by a relatively thin layer known as the thermocline.

b Ice cover and annual spring and fall overturns are due to successive seasonal changes in the relative densities of the epilimnion and the hypo-

limnion, profoundly influenced by prevailing meteorological conditions.

c The sudden exchange of water masses having different chemical characteristics may have catastrophic effects on certain biota.

d Silt laden waters may seek certain levels, depending on their own specific gravity in relation to existing layers already present.

e Saline waters will also stratify according to the relative densities of the various layers.

3 The viscosity of water is greater at lower temperatures.

a 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 and many delay the establishment of anchor ice under critical conditions.

b It is easier for plankton to remain suspended in cold viscous (and also dense) water than in less viscous warm water. This is reflected in differences in the appearance of winter vs summer forms of life (also arctic vs tropical).

D Shore development, depth, inflow - outflow pattern, and topographic features affect the behavior of the water.

E Water movements that may affect organisms include waves, currents, tides, seiches, and floods.

1 Waves or rhythmic movement

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

b Standing waves or seiches occur in all lakes but are seldom large enough to be observed. An "internal seich" is an oscillation in a density mass within a lake with no surface manifestation may cause considerable water movement.

2 Currents

a Currents are arhythmic water movements which have had major study only in oceanography. 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 runoff.

b 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.

F Surface Tension and the Surface Film

1 The surface film is the habitat of the "neuston", a group of particular importance in water supplies.

2 The biological effects of small amounts of detergents and similar agents are yet to be evaluated.

III DISSOLVED SUBSTANCES

A Carbon dioxide is released by plants

and animals in respiration, but taken in by plants in photosynthesis.

B Oxygen is the biological complement of carbon dioxide, and necessary for all animal life.

C Nitrogen and phosphorus are fundamental nutrients for plant life.

1 Occur in great dilution, concentrated by plants.

2 The distribution of nitrogen compounds is generally correlated with the oxygen curve.

D Iron, manganese, sulphur, and silicon are other minerals important to aquatic life which exhibit biological stratification.

E Many other minerals are present but their biological distribution in waters is less well known.

F Dissolved organic matter is present in even the purest of lakes.

V BIOLOGICAL FACTORS

A Nutritional Classification of Organisms

1 Holophytic or independent organisms, like green plants, produce their own basic food elements from the physical environment.

2 Holozoic or dependent organisms, like animals, ingest and digest solid food particles of organic origin.

3 Saprophytic or carrion eating organisms, like many fungi and bacteria, digest and assimilate the dead bodies of other organisms or their products.

B The Prey-Predator Relationship is Simply one Organism Eating Another.

C Toxic and Hormonic Relationships

1 Some organisms such as certain blue green algae and some armored flagellates produce substances poisonous to others.

2 Antibiotic action in nature is not well understood but has been shown to play a very influential role in the economy of nature.

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.

1 Those that pass through a plankton net (No. 25 silk bolting cloth or equivalent) or sand filter are known as nannoplankton (which usually greatly exceed the net plankton in quantity).

2 Those less than four microns in length are sometimes called ultraplankton.

3 There are many ways in which plankton may be classified: taxonomic, ecological, industrial.

4 The concentration of plankton varies markedly in space and time.

a Depth, light, currents, and water quality profoundly affect plankton distribution.

b The relative abundance of plankton in the various seasons is generally:

1 spring, 2 fall, 3 summer, 4 winter

B Benthic organisms (benthos) are those living on or near the bottom, frequently attached. Typically benthic organisms such as certain filamentous algae, may on occasion be broken or washed free,

and mingled with the typical plankton.

C The emergent vascular shore plants are often a very influential community; on death and decay, they add nutrients to the water.

D The shallow water or littoral community is one of the most varied and productive areas - a fluctuating water level will discourage this community.

E The deep water and sludge or ooze communities may contribute tastes, odors, and undesirable chemical characteristics.

VI THE EVOLUTION OF WATERS

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

B 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 streambed, 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 base level. During flood stage they scour their bed 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 as 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 Geological factors which significantly affect the nature of 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 responsive to many of the above factors and is also severely subject to the whims of civilization. This is one of the major factors determining run-off 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 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 irregulars areas.

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

d Rooted aquatics 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 sphagnum mats 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)

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. A productive water on the other hand may be a nuisance to man or highly desirable. Some of the factors which influence the productivity of waters are as follows:

B Factors affecting stream productivity. To be productive of plankton, 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.

3 Turbidity reduces productivity as light penetration is reduced.

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

5 Climate, temperature, prevalance 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 also important. The hypolimnion may may be anaerobic while the epilimnion is aerobic.

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.

VIII 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 menas of classification.

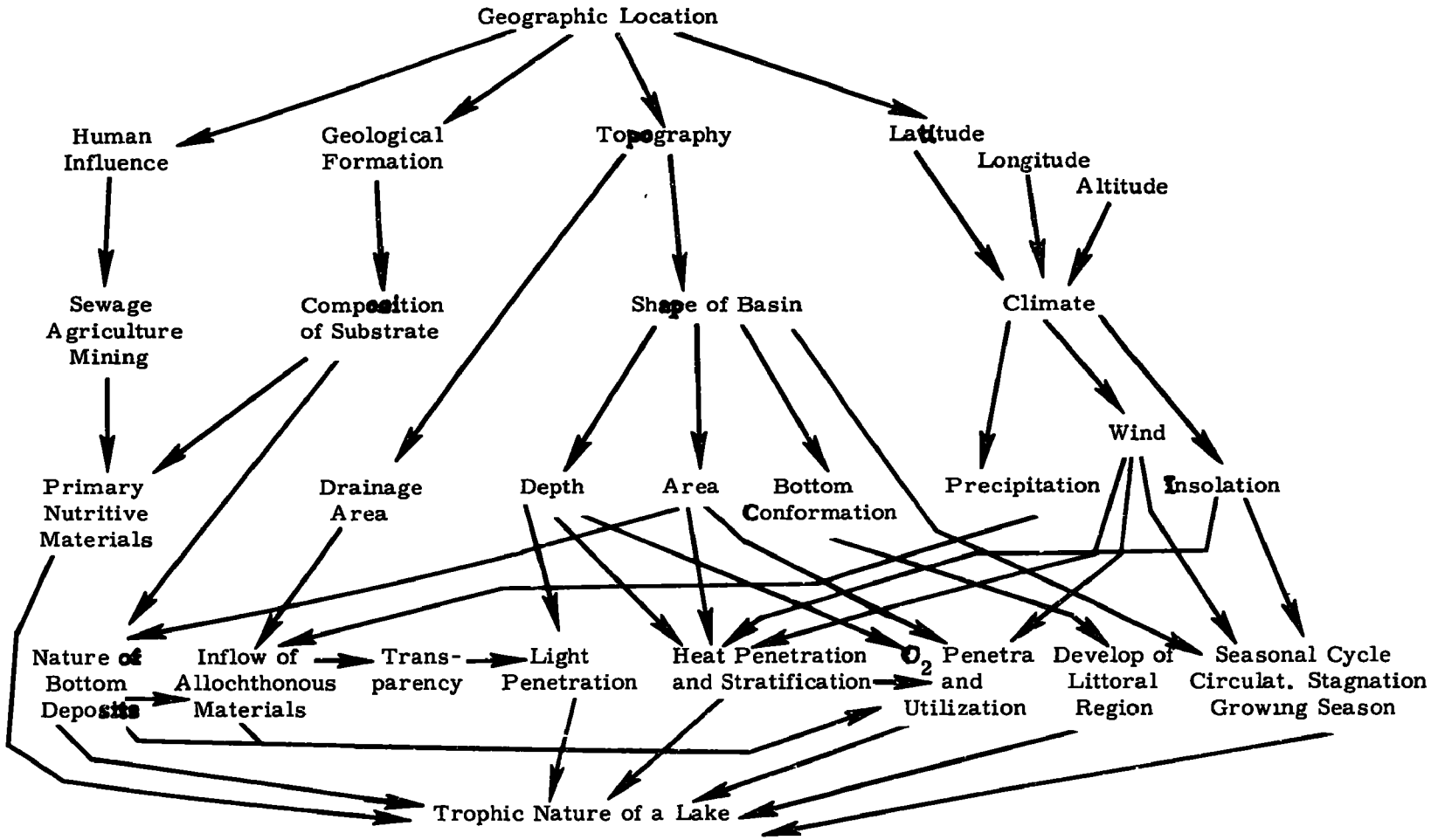
1 Oligotrophic lakes are the younger, less productive lakes, which are deep, have clear water, and usually support Salmonoid fishes in their deeper waters.

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 - bog lakes - 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 be classified into

FACTORS AFFECTING PRODUCTIVITY



two types, 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.

IX THE MANAGEMENT OR CONTROL OF ENVIRONMENTAL FACTORS

A Liebig's Law of the Minimum states that productivity is limited by the nutrient present in the least amoung at any given time relative to the assimilative capacity of the organism.

B Shelford's Law of Toleration:

Minimum Limit of toleration		Range of Optimum of factor	Maximum limit of toleration	
Absent	← Decreasing Abundance	Greatest abundance	→ Decreasing Abundance	Absent

C The artificial introduction of nutrients (sewage pollution or fertilizer) thus tneds to eliminate existing limiting minimums for some species and create intolerable maximums for other species.

1 Known limiting minimums may sometimes be deliberately maintained.

2 As the total available energy supply is increased, productivity tends to increase.

3 As productivity increases, the whole character of the water may be changed from a meagerly productive clear water lake (oligotrophic) to a highly productive and usually turbid lake (eutrophic).

4 Eutrophication leads to treatment troubles.

D Control of eutrophication may be accomplished by various means

1 Watershed management, adequate preparation of reservoir sites, and pollution control tend to maintain minimum limiting nutritional factors.

2 Shading out the energy of insolation by roofing or inert turbidity; suppresses photosynthesis.

3 Introduction of substances toxic to some fundamental part of the food chain (such as copper sulphate) tends to temporarily inhibit productivity.

X SUMMARY

A A body of water such as a lake 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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1 Chamberlin, Thomas C., and Salisburg, Rollin P., Geology Vol. 1, "Geological Processes and Their Results", pp 1-xix, and 1-654, Henry Holt and Company, New York, 1904.

I OPTICS

An understanding of elementary optics is essential to the proper use of the microscope. The microscopist will find that unusual problems in illumination and photomicrography can be handled much more effectively once the underlying ideas in physical optics are understood.

A Reflection

A good place to begin is with reflection at a surface or interface. Specular (or regular) reflection results when a beam of light leaves a surface at the same angle at which it reached it. This type of reflection occurs with highly polished smooth surfaces. It is stated more precisely as Snell's Law, i.e., the angle of incidence, i, is equal to the angle of reflection, r (Figure 1). Diffuse (or scattered) reflection results when a beam of light strikes a rough or irregular surface and different portions of the incident light are reflected from the surface at different angles. The light reflected from a piece of white paper or a ground glass is an example of diffuse reflection.

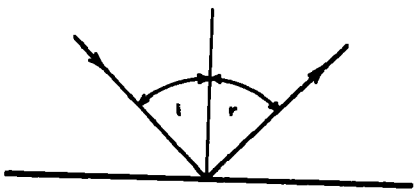

Figure 1

SPECULAR REFLECTION - SNELL'S LAW

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Strictly speaking, of course, all reflected light, even diffuse, obeys Snell's Law. Diffuse reflected light is made up of many specularly reflected rays, each from a a tiny element of surface, and appears diffuse when the reflecting elements are very numerous and very small. The terms diffuse and specular, referring to reflection, describe not so much a difference in the nature of the reflection but rather a difference in the type of surface. A polished surface gives specular reflection, a rough surface gives diffuse reflection.

It is also important to note and remember that specularly reflected light tends to be strongly polarized in the plane of the reflecting surface. This is due to the fact that those rays whose vibration directions lie closest to the plane of the reflection surface are most strongly reflected. This effect is strongest when the angle of incidence is such that the tangent of the angle is equal to the refractive index of the reflecting surface. This particular angle of incidence is called the Brewster angle.

B Image Formation on Reflection

Considering reflection by mirrors, we find (Figure 2) that a plane mirror forms a virtual image behind the mirror, reversed right to left but of the same size as the object. The word virtual means that the image appears to be in a given plane but that a ground glass screen or a photographic film placed in that plane would show no image. The converse of a virtual image is a real image.

Spherical mirrors are either convex or concave with the surface of the mirror representing a portion of the surface of a sphere. The center of curvature is the center of the sphere, part of whose surface forms the mirror. The focus lies halfway between the center of curvature and the mirror surface.

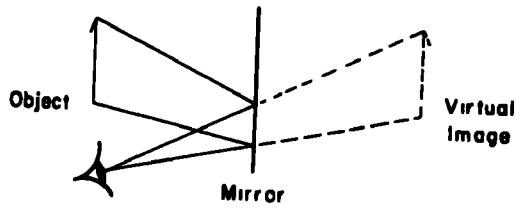


Figure 2

IMAGE FORMATION BY PLANE MIRROR

Construction of an image by a concave mirror follows from the two premises given below (Figure 3)·

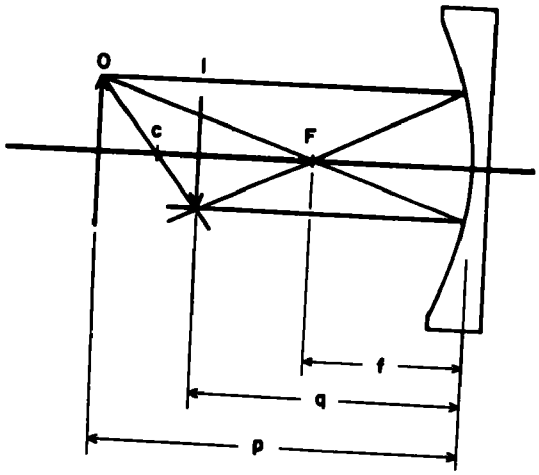


Figure 3

IMAGE FORMATION BY CONCAVE MIRROR

1 A ray of light parallel to the axis of the mirror must pass through the focus after reflection.

2 A ray of light which passes through the center of curvature must return along the same path.

A corollary of the first premise is:

3 A ray of light which passes through the focus is reflected parallel to the axis of the mirror.

The image from an object can be located using the familiar lens formula:

$$\frac{1}{p} + \frac{1}{q} \qquad \frac{1}{f}$$

where p = distance from the object to the mirror
q = distance from the image to the mirror
f = focal length

C Spherical Aberration

No spherical surface can be perfect in its image-forming ability. The most serious of the imperfections, spherical aberration, occurs in spherical mirrors of large aperture (Figure 4). The rays of light making up an image point from the outer zone of a spherical mirror do not pass through the same point as the more central rays. This type of aberration is reduced by blocking the outer zone rays from the image area or by using aspheric surfaces.

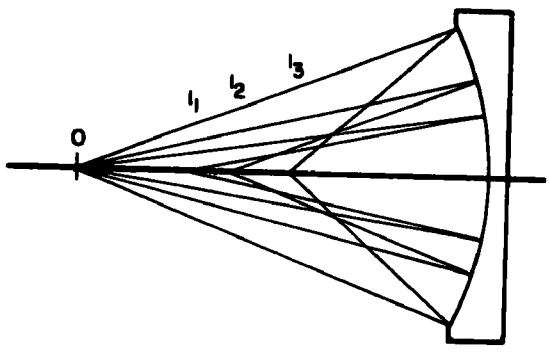


Figure 4

SPHERICAL ABERRATION BY SPHERICAL MIRROR

D Refraction of Light

Turning now to lenses rather than mirrors we find that the most important characteristic is refraction. Refraction refers to the change of direction and/or velocity of light as it passes from one medium to another. The ratio of the velocity in air (or more correctly in a vacuum) to the velocity in the medium is called the refractive index. Some typical values of refractive index measured with monochromatic light (sodium D line) are listed in Table 1.

Refraction causes an object immersed in a medium of higher refractive index than air to appear closer to the surface than it actually is (Figure 5). This effect may

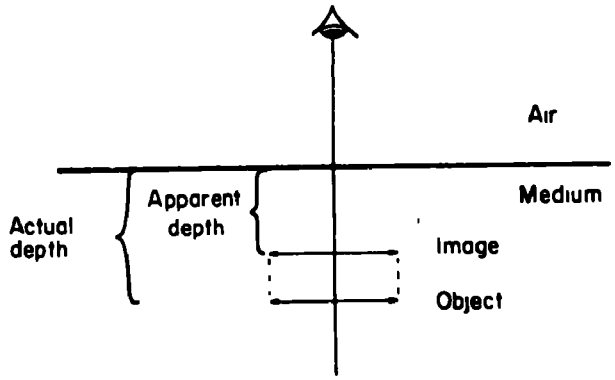


Figure 5
REFRACTION OF LIGHT AT INTERFACE

be used to determine the refractive index of a liquid with the microscope. A flat vial with a scratch on the bottom (inside) is placed on the stage of the microscope. The microscope is focused on the scratch and the fine adjustment micrometer reading is noted. A small amount of the unknown liquid is added; the scratch is again brought into focus and the new micrometer reading is taken. Finally, the microscope is refocused until the surface of the liquid appears in sharp focus. The micrometer reading is taken again and, with this information, the refractive index may be calculated from the simplified equation

$$\text{refractive index} = \frac{\text{actual depth}}{\text{apparent depth}}$$

Table 1 REFRACTIVE INDICES OF COMMON MATERIALS MEASURED WITH SODIUM LIGHT

Vacuum	1.0000000	Crown glass	1.48 to 1.61
Air	1.0002918	Rock salt	1.5443
CO_2	1.0004498	Diamond	2.417
Water	1.3330	Lead sulfide	3.912

When the situation is reversed, and a ray of light from a medium of high refractive index passes through the interface of a medium of lower index, the ray is refracted until a critical angle is reached beyond which all of the light is reflected from the interface (Figure 6). This critical angle, C, has the following relationship to the refractive indices of the two media

$$\sin C = \frac{n_2}{n_1}, \text{ where } n_2 < n_1.$$

When the second medium is air, the formula becomes

$$\sin C = \frac{1}{n_1}.$$

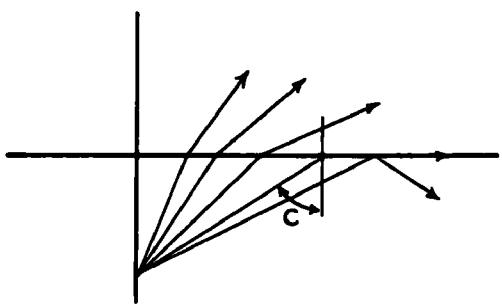


Figure 6
REFLECTION AT CRITICAL ANGLE

E Dispersion

Dispersion is another important property of transparent materials. This is the variation of refractive index with color (or wavelength) of light. When white light passes through a glass prism, the light rays are refracted by different amounts and separated into the colors of the spectrum. This spreading of light into its component colors is due to dispersion which, in turn, is due to the fact that the refractive index of transparent substances, liquids and solids, is lower for long wavelengths than for short wavelengths.

Because of dispersion, determination of the refractive index of a substance requires designation of the particular wavelength used. Light from a sodium lamp has a strong, closely spaced doublet with an average wavelength of 5893A, called the D line, which is commonly used as a reference wavelength. Table 2 illustrates the change of refractive index with wavelength for a few common substances.

Table 2. DISPERSION OF REFRACTIVE INDICES OF SEVERAL COMMON MATERIALS

	Refractive index		
	F line blue 4861A	D line (yellow) 5893A	C line (red) 6563A
Carbon disulfide	1.6523	1.6276	1.6182
Crown glass	1.5240	1.5172	1.5145
Flint glass	1.6391	1.6270	1.6221
Water	1.3372	1.3330	1.3312

The dispersion of a material can be defined quantitatively as:

$$\nu = \text{dispersion} = \frac{n\,(\text{yellow}) - 1}{n\,(\text{blue}) - n\,(\text{red})}$$

$$= \frac{n\,(593m\mu) - 1}{n\,(486m\mu) - n(656m\mu)}$$

where n is the refractive index of the material at the particular wavelength noted in the parentheses.

F Lenses

There are two classes of lenses, converging and diverging, called also convex and concave, respectively. The focal point of a converging lens is defined as the point at which a bundle of light rays parallel to the axis of the lens appears to converge after passing through the lens. The focal length of the lens is the distance from the lens to the focal point (Figure 7).

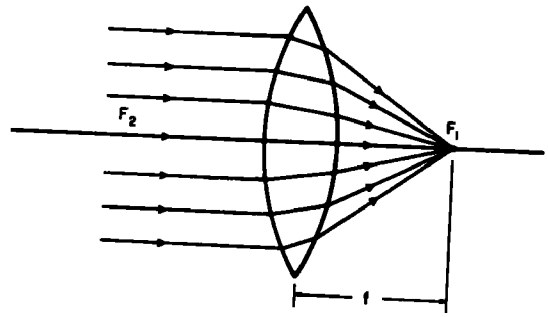


Figure 7

CONVERGENCE OF LIGHT AT FOCAL POINT

G Image Formation by Refraction

Image formation by lenses (Figure 8) follows rules analogous to those already given above for mirrors

1 Light traveling parallel to the axis of the lens will be refracted so as to pass through the focus of the lens.

2 Light traveling through the geometrical center of the lens will be unrefracted.

The position of the image can be determined by remembering that a light ray passing through the focus, F, will be parallel to the axis of the lens on the opposite side of the lens and that a ray passing through the geometrical center of the lens will be unrefracted.

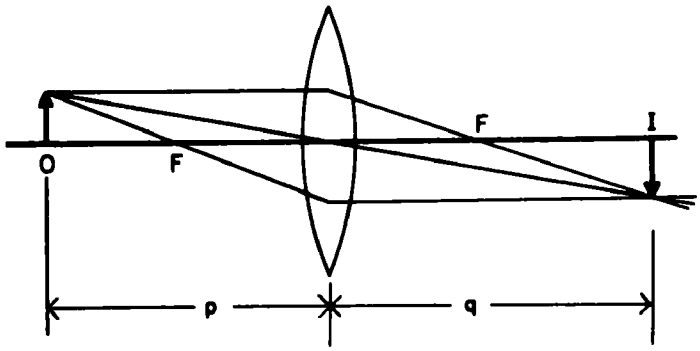


Figure 8

IMAGE FORMATION BY A CONVEX LENS

The magnification, M, of an image of an object produced by a lens is given by the relationship:

$$M = \frac{\text{image size}}{\text{object size}} = \frac{\text{image distance}}{\text{object distance}} = \frac{q}{p}$$

where q = distance from image to lens
and p = distance from object to lens.

H Aberrations of Lenses

Lenses have aberrations of several types which, unless corrected, cause loss of detail in the image. Spherical aberration appears in lenses with spherical surfaces. Reduction of spherical aberration can be accomplished by diaphragming the outer zones of the lens or by designing special aspherical surfaces in the lens system.

Chromatic aberration is a phenomenon caused by the variation of refractive index with wavelength (dispersion). Thus a lens receiving white light from an object will form a violet image closer to the lens and a red one farther away. Achromatic lenses are employed to minimize this effect. The lenses are combinations of two or more lens elements made up of materials having different dispersive powers. The use of monochromatic light is another obvious way of eliminating chromatic aberration.

Astigmatism is a third aberration of spherical lens systems It occurs when object points are not located on the optical axis of the lens and results in the formation of an indistinct image. The simplest remedy for astigmatism is to place the object close to the axis of the lens system.

I Interference Phenomena

Interference and diffraction are two phenomena which are due to the wave characteristics of light. The superposition of two light rays arriving simultaneously at a given point will give rise to interference effects, whereby the intensity at that point will vary from dark to bright depending on the phase differences between the two light rays.

The first requirement for interference is that the light must come from a single source. The light may be split into any number of paths but must originate from the same point (or coherent source). Two light waves from a coherent source arriving at a point in phase agreement will reinforce each other (Figure 9a). Two light waves from a coherent source arriving at a point in opposite phase will cancel each other (Figure 9b).

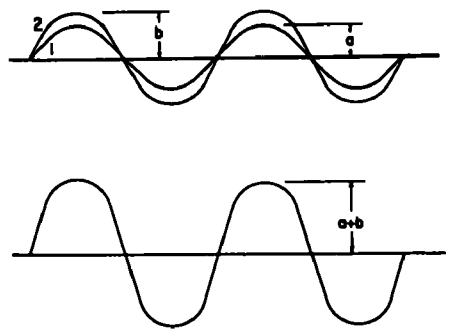


Figure 9a. Two light rays, 1 and 2, of the same frequency but different amplitudes, are in phase in the upper diagram. In the lower diagram, rays 1 and 2 interfere constructively to give a single wave of the same frequency and with an amplitude equal to the summation of the two former waves.

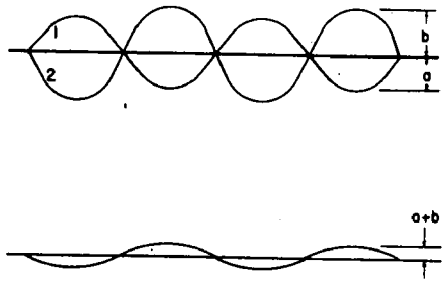


Figure 9b. Rays 1 and 2 are now 180° out of phase and interfere destructively. The resultant, in the bottom diagram, is of the same frequency but is of reduced amplitude (a is negative and is subtracted from b).

The reflection of a monochromatic light beam by a thin film results in two beams, one reflected from the top surface and one from the bottom surface. The distance traveled by the latter beam in excess of the first is twice the thickness of the film and its equivalent air path is:

$$2\,nt$$

where n is the refractive index and t is the thickness of the film.

The second beam, however, upon reflection at the bottom surface, undergoes a half wavelength shift and now the total retardation of the second beam with respect to the first is given as:

$$\text{retardation} = 2\,nt + \frac{\lambda}{2}$$

where λ is the wavelength of the light beam.

When retardation is exactly an odd number of half wavelengths, destructive interference takes place resulting in darkness. When it is zero or an even number of half wavelengths, constructive interference results in brightness (Figure 10).

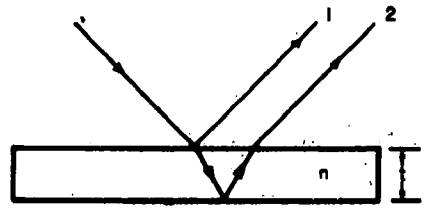


Figure 10

INTERFERENCE IN A THIN FILM

A simple interferometer can be made by partially silvering a microscope slide and cover slip. A preparation between the two partially silvered surfaces will show interference fringes when viewed with monochromatic light, either transmitted or by vertical illuminator. The fringes will be close together with a wedge-shaped preparation and will reflect refractive index differences due to temperature variations, concentration differences, different solid phases, etc. The method has been used to measure quantitatively the concentration of solute around a growing crystal[1] (Figure 11).

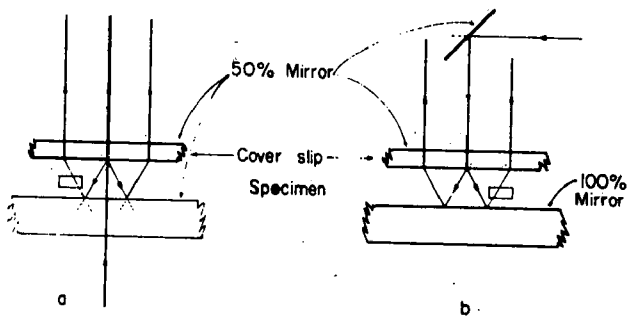


Figure 11

MICROSCOPICAL METHOD OF VIEWING INTERFERENCE IMAGES

a Examination is by transmitted light. Light ray undergoes multiple reflections and produces dark and light fringes in the field. A specimen introduces a phase shift and changes the fringe pattern.

b Illumination is from the top. The principle is the same but fringes show greater contrast.

Each dark band represents an equivalent air thickness of an odd number of half wavelengths. Conversely, each bright band is the result of an even number of half wavelengths.

With interference illumination, the effect of a transparent object of different refractive index than the medium in the microscope field is:

1 a change of light intensity of the object if the background is uniformly illuminated (parallel cover slip), or

2 a shift of the interference bands within the object if the background consists of bands (tilted cover slip).

The relationship of refractive indices of the surrounding medium and the object is as follows:

$$n_s = n_m\left(1 + \frac{\theta\lambda}{360t}\right)$$

where n_s = refractive index of the specimen
n_m = refractive index of the surrounding medium
θ = phase shift of the two beams, degrees
λ = wavelength of the light
t = thickness of the specimen.

J Diffraction

In geometrical optics, it is assumed that light travels in straight lines. This is not always true. We note that a beam passing through a slit toward a screen creates a bright band wider than the slit with alternate bright and dark bands appearing on either side of the central bright band, decreasing in intensity as a function of the distance from the center. Diffraction describes this phenomenon and, as one of its practical consequences, limits the lens in its ability to reproduce an image. For example, the image of a pin point of light produced by a lens is not a pin point but is revealed to be a somewhat larger patch of light surrounded by dark and bright rings. The diameter, d, of this diffraction disc (to the first dark ring) is given as:

$$d = \frac{2.44\, f\lambda}{D}$$

where f is the focal length of the lens, λ the wavelength, and D the diameter of the lens.

It is seen that in order to maintain a small diffraction disc at a given wavelength, the diameter of the lens should be as large as possible with respect to the focal length. It should be noted, also, that a shorter wavelength produces a smaller disc.

If two pin points of light are to be distinguished in an image, their diffraction discs must not overlap more than one half their diameters. The ability to distinguish such image points is called resolving power and is expressed as one half of the preceding expression

$$\text{resolving power} = \frac{1.22\, f\lambda}{D}.$$

II THE COMPOUND MICROSCOPE

The compound microscope is an extension in principle of the simple magnifying glass, hence it is essential to understand fully the properties of this simple lens system.

A Image Formation by the Simple Magnifier

The apparent size of an object is determined by the angle that is formed at the eye by the extreme rays of the object. By bringing the object closer to the eye, that angle (called the visual angle) is increased. This also increases the apparent size. However a limit of accommodation of the eye is reached, at which distance the eye can no longer focus. This limiting distance is about 10 inches or 25 centimeters. It is at this distance that the magnification of an object observed by the unaided eye is said to be unity. The eye can, of course, be focused at shorter distances but not usually in a relaxed condition.

A positive, or converging, lens can be used to permit placing an object closer than 10 inches to the eye (Figure 12). By this means the visual angle of the object is increased (as is its apparent size) while the image of

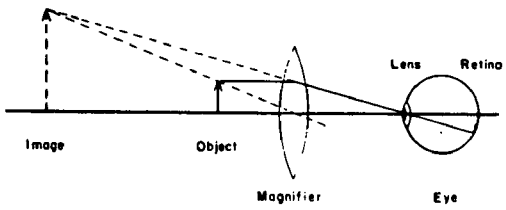


Figure 12

VIRTUAL IMAGE FORMATION BY CONVEX LENS

the object appears to be 10 inches from the eye, where it is best accommodated.

B Magnification by a Single Lens System

The magnification, M, of a simple magnifying glass is given by:

$$M = \frac{25}{f} + 1$$

where f = focal length of the lens in centimeters.

Theoretically the magnification can be increased with shorter focal length lenses. However such lenses require placing the eye very close to the lens surface and have much image distortion and other optical aberrations. The practical limit for a simple magnifying glass is about 20X.

In order to go to magnifications higher than 20X, the compound microscope is required. Two lens systems are used to form an enlarged image of an object (Figure 13). This is accomplished in two steps, the first by a lens called the objective and the second by a lens known as the eyepiece (or ocular).

C The Objective

The objective is the lens (or lens system) closest to the object. Its function is to reproduce an enlarged image of the object in the body tube of the microscope. Objectives are available in various focal

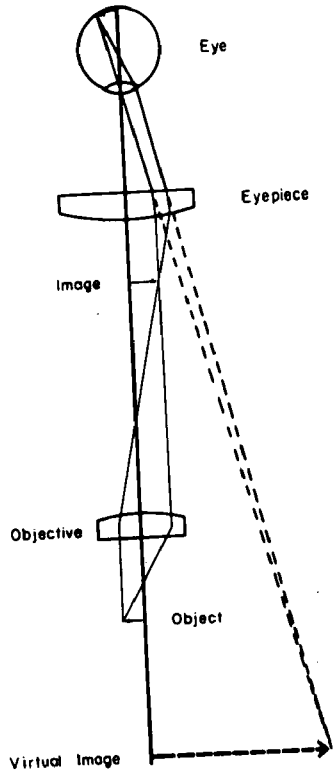


Figure 13

IMAGE FORMATION IN COMPOUND MICROSCOPE

lengths to give different magnifications (Table 3). The magnification is calculated from the focal length by dividing the latter into the tube length, usually 160 mm.

The numerical aperture (N.A.) is a measure of the ability of an objective to resolve detail. This is more fully discussed in the next section. The working distance is in the free space between the objective and the cover slip and varies slightly for objectives of the same focal length depending upon the degree of correction and the manufacturer.

There are three basic classifications of objectives: achromats, fluorites and apochromats, listed in the order of their complexity. The achromats are good for routine work while the fluorites and apochromats offer additional optical corrections to compensate for spherical, chromatic and other aberrations.

Table 3. NOMINAL CHARACTERISTICS OF USUAL MICROSCOPE OBJECTIVES

Nominal focal length mm	Nominal magnif.	N.A.	Working distance mm	Depth focus μ	Diam. of field mm.	Resolving power, white light, μ	Maximum useful magnif.	Eyepiece for max. useful magnif.
56	2.5X	0.08	40	50	8.5	4.4	80X	30X
32	5	0.10	25	16	5	3.9	90X	20X
16	10	0.25	7	8	2	1.4	250X	25X
8	20	0.50	1.3	2	1	0.7	500X	25X
4	43	0.66	0.7	1	0.5	0.4	660X	15X
4	45	0.85	0.5	1	0.4	0.35	850X	20X
1.8	90	1.30	0.2	0.4	0.2	0.21	1250X	12X

Another system of objectives employs reflecting surfaces in the shape of concave and convex mirrors. Reflection optics, because they have no refracting elements, do not suffer from chromatic aberrations as ordinary refraction objectives do. Based entirely on reflection, reflecting objectives are extremely useful in the infrared and ultraviolet regions of the spectrum. They also have a much longer working distance than the refracting objectives.

The body tube of the microscope supports the objective at the bottom (over the object) and the eyepiece at the top. The tube length is maintained at 160 mm except for Leitz instruments, which have a 170-mm tube length.

The objective support may be of two kinds, an objective clutch changer or a rotating nosepiece

1 The objective clutch changer ("quick-change" holder) permits the mounting of only one objective at a time on the microscope. It has a centering arrangement, so that each objective need be centered only once with respect to the stage rotation. The changing of objectives with this system is somewhat awkward compared with the rotating nosepiece.

2 The revolving nosepiece allows mounting three or four objectives on the microscope at one time (there are some nosepieces that accept five and even six objectives). In this system, the objectives are usually noncenterable and the stage is centerable. Several manufacturers provide centerable objective mounts so that each objective on the nosepiece need be centered only once to the fixed rotating stage. The insides of objectives are better protected from dust by the rotating nosepiece. This, as well as the inconvenience of the so-called "quick-change" objective holder, makes it worthwhile to have one's microscope fitted with rotating nosepiece.

D The Ocular

The eyepiece, or ocular, is necessary in the second step of the magnification process. The eyepiece functions as a simple magnifier viewing the image formed by the objective.

There are three classes of eyepieces in common use: huyghenian, compensating and flat-field. The huyghenian (or huyghens) eyepiece is designed to be used with achromats while the compensating type is used with fluorite and apochromatic objectives. Flat-field eyepieces, as the name implies, are employed in photomicrography or projection and can be used with most objectives. It is best to follow the recommendations of the manufacturer as to the proper combination of objective and eyepiece.

The usual magnifications available in oculars run from about 6X up to 25 or 30X. The 6X is generally too low to be of any real value while the 25 and 30X oculars have slightly poorer imagery than medium powers and have a very low eyepoint. The most useful eyepieces lie in the 10 to 20X magnification range.

E Magnification of the Microscope

The total magnification of the objective-eyepiece combination is simply the product of the two individual magnifications. A convenient working rule to assist in the proper choice of eyepieces states that the maximum useful magnification (MUM) for the microscope is 1,000 times the numerical aperture (N.A.) of the objective. The MUM is related to resolving power in that magnification in excess of MUM gives little or no additional resolving power and results in what is termed empty magnification. Table 4 shows the results of such combinations and a comparison with the 1000X N.A. rule. The underlined figure shows the magnification nearest to the MUM and the eyepiece required with each objective to achieve the MUM. From this table it is apparent that only higher power eyepieces can give full use of the resolving power of the objectives. It is obvious that a 10X, or even a 15X, eyepiece gives insufficient magnification for the eye to see detail actually resolved by the objective.

F Focusing the Microscope

The coarse adjustment is used to roughly position the body tube (in some newer microscopes, the stage) to bring the image into focus. The fine adjustment is used after the coarse adjustment to bring the image into perfect focus and to maintain the focus as the slide is moved across the stage. Most microscope objectives are parfocal so that once they are focused any other objective can be swung into position without the necessity of refocusing except with the fine adjustment.

The student of the microscope should first learn to focus in the following fashion, to prevent damage to a specimen or objective:

1 Raise the body tube and place the specimen on the stage.

2 Never focus the body tube down (or the stage up) while observing the field through the eyepiece.

3 Lower the body tube (or raise the stage) with the coarse adjustment while carefully observing the space between the

Table 4. MICROSCOPE MAGNIFICATION CALCULATED FOR VARIOUS OBJECTIVE-EYEPIECE COMBINATIONS

Objective		Eyepiece					MUM[a]
Focal length	Magnification	5X	10X	15X	20X	25X	(1000 NA)
56mm	3X	15X	30X	45X	60X	<u>75X</u>	80X
32	5	25X	50X	75X	<u>100X</u>	125X	100X
16	10	50X	100X	150X	200X	<u>250X</u>	250X
8	20	100X	200X	300X	400X	<u>500X</u>	500X
4	40	200X	400X	600X	<u>800X</u>	1000X	660X
1.8	90	450X	900X	<u>1350X</u>	1800X	2250X	1250X

[a]MUM = maximum useful magnification

objective and slide and permitting the two to come close together without touching.

4 Looking through the microscope and turning the fine adjustment in such a way as to move the objective away from the specimen, bring the image into sharp focus.

The fine adjustment is usually calibrated in one- or two-micron steps to indicate the vertical movement of the body tube. This feature is useful in making depth measurements but should not be relied upon for accuracy.

G The Substage Condenser

The substage holds the condenser and polarizer. It can usually be focused in a vertical direction so that the condenser can be brought into the correct position with respect to the specimen for proper illumination. In some models, the condenser is centerable so that it may be set exactly in the axis of rotation of the stage, otherwise it will have been precentered at the factory and should be permanent.

H The Microscope Stage

The stage of the microscope supports the specimen between the condenser and objective, and may offer a mechanical stage as an attachment to provide a means of moving the slide methodically during observation. The polarizing microscope is fitted with a circular rotating stage to which a mechanical stage may be added. The rotating stage, which is used for object orientation to observe optical effects, will have centering screws if the objectives are not centerable, or vice versa. It is undesirable to have both objectives and stage centerable as this does not provide a fixed reference axis.

I The Polarizing Elements

A polarizer is fitted to the condenser of all polarizing microscopes. In routine instruments, the polarizer is fixed with its vibration direction oriented north-south (east-west for most European instruments) while in research microscopes, the polarizer can be rotated. Modern instruments have polarizing filters (such as Polaroid) replacing the older calcite prisms. Polarizing filters are preferred because they

1 are low-cost,

2 require no maintenance,

3 permit use of the full condenser aperture

An analyzer, of the same construction as the polarizer, is fitted in the body tube of the microscope on a slider so that it may be easily removed from the optical path. It is oriented with its plane of vibration perpendicular to the corresponding direction of the polarizer.

J The Bertrand Lens

The Bertrand lens is usually found only on the polarizing microscope although some manufacturers are beginning to include it on phase microscopes. It is located in the body tube above the analyzer on a slider (or pivot) to permit quick removal from the optical path. The Bertrand lens is used to observe the back focal plane of the objective. It is convenient for checking quickly the type and quality of illumination, for observing interference figures of crystals, for adjusting the phase annuli in phase microscopy and for adjusting the annular and central stops in dispersion staining.

K The Compensator Slot

The compensator slot receives compensators (quarter-wave, first-order red and quartz-wedge) for observation of the optical properties of crystalline materials. It is usually placed at the lower end of the body tube just above the objective mount, and is oriented 45° from the vibration directions of the polarizer and analyzer.

L The Stereoscopic Microscope

The stereoscopic microscope, also called the binocular, wide-field, dissecting or

Greenough binocular microscope, is in reality a combination of two separate compound microscopes. The two microscopes, usually mounted in one body, have their optical axes inclined from the vertical by about 7° and from each other by twice this angle. When an object is placed on the stage of a stereoscopic microscope, the optical systems view it from slightly different angles, presenting a stereoscopic pair of images to the eyes, which fuse the two into a single three-dimensional image.

The objectives are supplied in pairs, either as separate units to be mounted on the microscope or, as in the new instruments, built into a rotating drum. Bausch and Lomb was the first manufacturer to have a zoom lens system which gives a continuous change in magnification over the full range. Objectives for the stereomicroscope run from about 0.4X to 12X, well below the magnification range of objectives available for single-objective microscopes.

The eyepieces supplied with stereoscopic microscopes run from 10 to 25X and have wider fields than their counterparts in the single-objective microscopes.

Because of mechanical limitations, the stereomicroscope is limited to about 200X magnification and usually does not permit more than about 120X. It is most useful at relatively low powers in observing shape and surface texture, relegating the study of greater detail to the monocular microscope. The stereomicroscope is also helpful in manipulating small samples, separating ingredients of mixtures, preparing specimens for detailed study at higher magnifications and performing various mechanical operations under microscopical observation, e. g. micromanipulation.

III ILLUMINATION AND RESOLVING POWER

Good resolving power and optimum specimen contrast are prerequisites for good microscopy. Assuming the availability of suitable optics (ocular, objectives and substage condenser) it is still of paramount importance to use proper illumination. The requirement for a good illumination system for the microscope is to have uniform intensity of illumination over the entire field of view with independent control of intensity and of the angular aperture of the illuminating cone.

A Basic Types of Illumination

There are three types of illumination (Table 5) used generally.

1 Critical. This is used when high levels of illumination intensity are necessary for oil immersion, darkfield, fluorescence, low birefringence or photomicrographic studies. Since the lamp filament is imaged in the plane of the specimen, a ribbon filament or arc lamp is required. The lamp must be focusable and have an iris diaphragm, the position of the filament must also be adjustable in all directions.

2 Köhler. Also useful for intense illumination, Köhler illumination may be obtained with any lamp not fitted with a ground glass. The illuminator must, however, be focusable, it must have an adjustable field diaphragm (iris) and the lamp filament position must be adjustable in all directions.

3 "Poor man's". So-called because a low-priced illuminator may be used, this method gives illumination of high quality although of lower intensity because of the presence of a ground glass in the system. No adjustments are necessary on the illuminator or lamp filament although an adjustable diaphragm on the illuminator is helpful.

All three types of illumination require that the microscope substage condenser focus the image of the illuminator aperture in the plane of the specimen. In each case, then, the lamp iris acts as a field diaphragm and should be closed to just illuminate the field of view. The differences in these three types of illumination lie in the adjustment of the lamp condensing lens. With poor man's illumination there is no lamp condenser, hence no adjustment. The lamp should be placed close to the microscope so that

Table 5. COMPARISON OF CRITICAL, KÖHLER AND POOR MAN'S ILLUMINATION

	Critical	Köhler	Poor man's
Lamp filament	ribbon filament	any type	any type
Lamp condensing lens	required	required	none
Lamp iris	required	required	useful
Ground glass at lamp	none	none	present
Image of light source	in object plane	at substage iris	none
Image of field iris	near object plane	in object plane	near object plane
Image of substage iris	back focal plane of objective	back focal plane of objective	back focal plane of objective

the entire field of view is always illuminated. If the surface structure of the ground glass becomes apparent in the field of view the substage condenser is very slightly defocused.

Critical Illumination

With critical illumination the lamp condenser is focused to give parallel rays; focusing the lamp filament on a far wall is sufficient. Aimed, then, at the substage mirror, the substage condenser will focus the lamp filament in the object plane. The substage condenser iris will now be found imaged in the back focal plane of the objective; it serves as a control over convergence of the illumination. Although the substage iris also affects the light intensity over the field of view it should most decidedly not be used for this purpose. The intensity of illumination may be varied by the use of neutral density filters and, unless color photomicrography is anticipated, by the use of variable voltage on the lamp filament.

Köhler illumination (Figure 14) differs from critical illumination in the use of the lamp condenser. With critical illumination the lamp condenser focuses the lamp filament at infinity, with Köhler illumination the lamp filament is focused in the plane of the substage condenser iris (also coincident with the anterior focal plane of the substage condenser). The functions of the lamp condenser iris and the substage condenser iris in controlling, respectively, the area of the illuminated field of view and the angular aperture of the illuminating cone are precisely alike for all three types of illumination.

Critical illumination is seldom used because it requires a special lamp filament and because, when used, it shows no advantage over well-adjusted Köhler illumination.

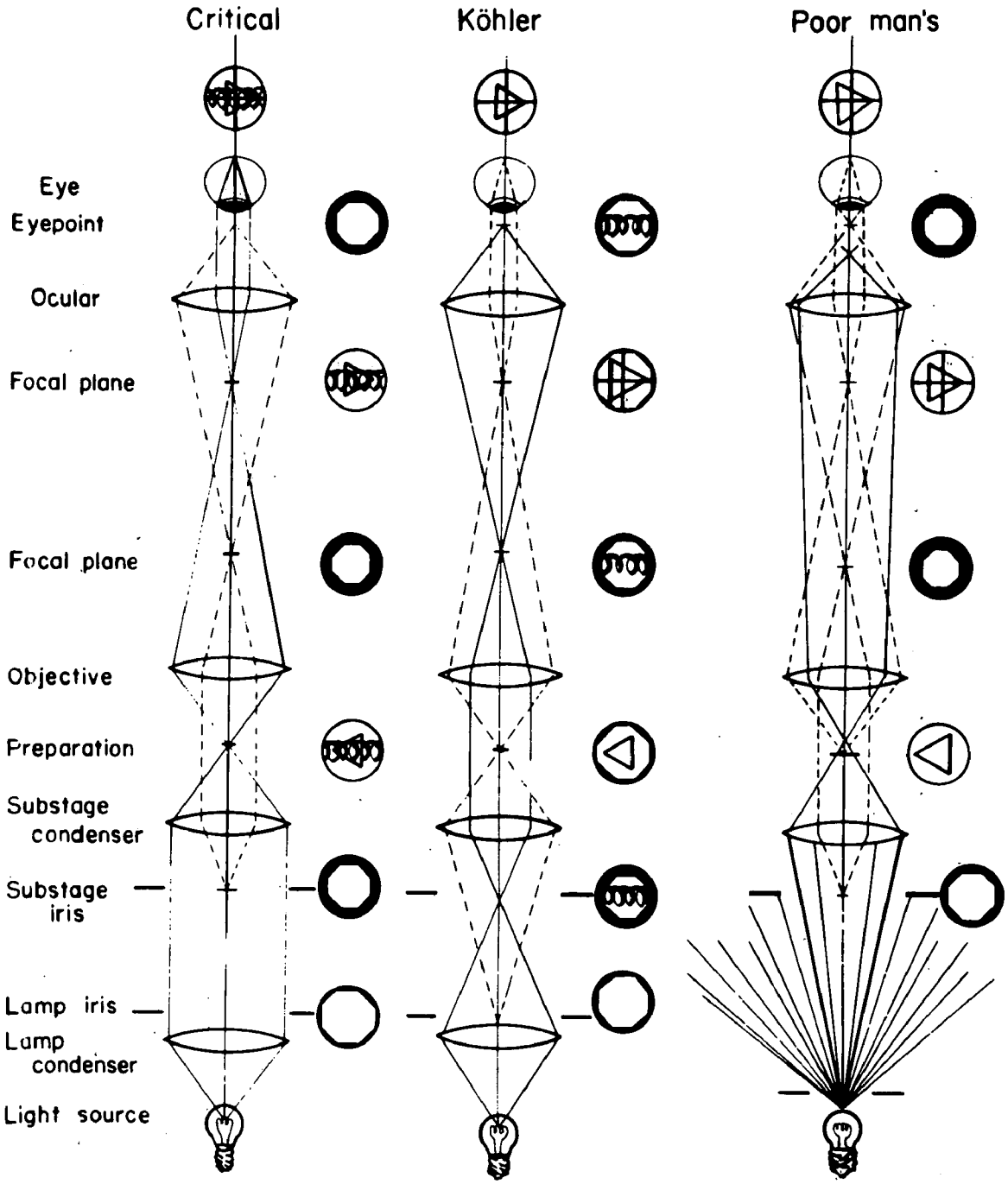
Köhler Illumination

To arrange the microscope and illuminator for Köhler illumination it is well to proceed through the following steps:

a Remove the diffusers and filters from the lamp.

b Turn the lamp on and aim at a convenient wall or vertical screen about 19 inches away. Open the lamp diaphragm.

c By moving the lamp condenser, focus a sharp image of the filament. It should be of such a size as to fill, not necessarily evenly, the microscope


Critical
Köhler
Poor man's
Eye
Eyepoint
Ocular
Focal plane
Focal plane
Objective
Preparation
Substage condenser
Substage iris
Lamp iris
Lamp condenser
Light source

substage condenser opening. If it does not, move the lamp away from the wall to enlarge the filament image, refocus.

d Turn the lamp and aim it at the microscope mirror so as to maintain the same 18 inches (or adjusted lamp distance).

e Place a specimen on the microscope stage and focus sharply with a 16-mm (10X) objective. Open fully the aperture diaphragm in the substage condenser. If the light is too bright, temporarily place a neutral density filter or a diffuser in the lamp.

f Close the lamp diaphragm, or field diaphragm, to about a 1-cm opening. Rack the microscope substage condenser up and down to focus the field diaphragm sharply in the same plane as the specimen.

g Adjust the mirror to center the field diaphragm in the field of view.

h Remove the 16-mm objective and replace with a 4-mm objective. Move the specimen so that a clear area is under observation. Place the Bertrand lens in the optical path, or remove the eyepiece and insert an auxiliary telescope (sold with phase contrast accessories) in its place, or remove the eyepiece and observe the back aperture of the objective directly. Remove any ground glass diffusers from the lamp. Now observe the lamp filament through the microscope.

i If the filament does not appear to be centered, swing the lamp housing in a horizontal arc whose center is at the field diaphragm. The purpose is to maintain the field diaphragm on the lamp in its centered position. If a vertical movement of the filament is required, loosen the bulb base and slide it up or down. If the base is fixed, tilt the lamp housing in a vertical arc with the field diaphragm as the center of movement (again endeavoring to keep the lamp diaphragm in the centered position). If you have mastered this step, you have accomplished the most difficult portion. (Better microscope lamps have adjustments to move the bulb independently of the lamp housing to simplify this step.)

j Put the specimen in place, replace the eyepiece and the desired objective and refocus.

k Open or close the field diaphragm until it just disappears from the field.

l Observe the back aperture of the objective, preferably with the Bertrand lens or the auxiliary telescope, and close the aperture diaphragm on the substage condenser until it is about four-fifths the diameter of the back aperture. This is the best position for the aperture diaphragm, a position which minimizes glare and maximizes the resolving power. It is instructive to vary the aperture diaphragm and observe the image critically during the manipulation.

m If the illumination is too great, insert an appropriate neutral density filter between the illuminator and the condenser. Do not use the condenser aperture diaphragm or the lamp field diaphragm to control the intensity of illumination.

Poor Man's Illumination

Both critical and Köhler illumination require expensive illuminators with adjustable focus, lamp iris and adjustable lamp mounts. Poor man's illumination requires a cheap illuminator although an expensive illuminator may be used if its expensive features are negated by inserting a ground glass diffuser or by using a frosted bulb. Admittedly an iris diaphragm on the lamp would be a help though it is not necessary.

a The illuminator must have a frosted bulb or a ground glass diffuser.

It should be possible to direct it in the general direction of the substage mirror, very close thereto or in place thereof.

b Focus on any preparation after tilting the mirror to illuminate the field.

c Remove the top lens of the condenser and, by racking the condenser up or, more often, down, bring into focus (in the same plane as the specimen) a finger, pencil or other object placed in the same general region as the ground glass diffuser on the lamp. The glass surface itself can then be focused in the plane of the specimen.

d Ideally the ground glass surface will just fill the field of view when centered by the substage mirror, adjustment may be made by moving the lamp closer to or farther from the microscope (the position might be marked for each objective used) or by cutting paper diaphragms of fixed aperture (one for each objective used). In this instance a lamp iris would be useful.

e Lower the condenser just sufficiently to defocus the ground glass surface and render the field of illumination even.

f Observe the back aperture of the objective and open the substage condenser iris about 75 percent of the way. The final adjustment of the substage iris is made while observing the preparation, the iris should be open as far as possible, still giving good contrast.

g The intensity of illumination should be adjusted only with neutral density filters or by changing the lamp voltage.

Proper illumination is one of the most important operations in microscopy. It is easy to judge a microscopist's ability by a glance at his field of view and the objective back lens.

B Resolving Power

The resolving power of the microscope is its ability to distinguish separate details of closely spaced microscopic structures. The theoretical limit of resolving two discrete points, a distance X apart, is

$$X \quad \frac{1.22\lambda}{2\,N.A.}$$

where λ = wavelength of light used to illuminate the specimen
N.A. = numerical aperture of the objective

Substituting a wavelength of 4,500 Angstroms and a numerical aperture of 1.3, about the best that can be done with visible light, we find that two points about 2,000A (or 0.2 micron) apart can be seen as two separate points. Further increase in resolving power can be achieved for the light microscope by using light or shorter wavelength. Ultraviolet light near 2,000 Angstroms lowers the limit to about 0.1 micron, the lower limit for the light microscope.

The numerical aperture of an objective is usually engraved on the objective and is related to the angular aperture, AA (Figure 15), by the formula:

$$N.A. = n \sin \frac{AA}{2}$$

where n = the lowest index in the space between the object and the objective.

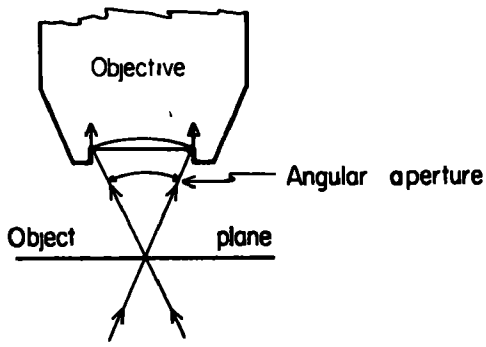


Figure 15

ANGULAR APERTURE OF MICROSCOPE OBJECTIVE

1 Maximum useful magnification

A helpful rule of thumb is that the useful magnification will not exceed 1,000 times the numerical aperture of the objective (see Tables 3 and 4). Although somewhat higher magnification may be used in specific cases, no additional detail will be resolved.

It is curious, considering the figures in the table, that most, if not all, manufacturers of microscopes furnish a 10X eyepiece as the highest power. A 10X eyepiece is useful but anyone interested in critical work should use a 15-25X eyepiece; the 5-10X eyepieces are best for scanning purposes.

2 Abbe's theory of resolution

One of the most cogent theories of resolution is due to Ernst Abbe, who suggested that microscopic objects act like diffraction gratings (Figure 16) and that the angle of diffraction, therefore, increases with the fineness of the detail. He proposed that a given microscope objective would resolve a particular detail if at least two or three transmitted rays (one direct and two diffracted rays) entered the objective. In Figure 16 the detail shown would be resolved in A and C but not in B. This theory, which can be borne out by simple experiment, is useful in showing how to improve resolution. Since shorter wavelengths will give a smaller diffraction angle, there is more chance of resolving fine detail with short wavelengths. Also, since only two of the transmitted rays are needed, oblique light and a high N.A. condenser will aid in resolving fine detail.

3 Improving resolving power

The following list summarizes the practical approaches to higher resolution with the light microscope:

a The specimen should be illuminated by either critical or Kohler illumination.

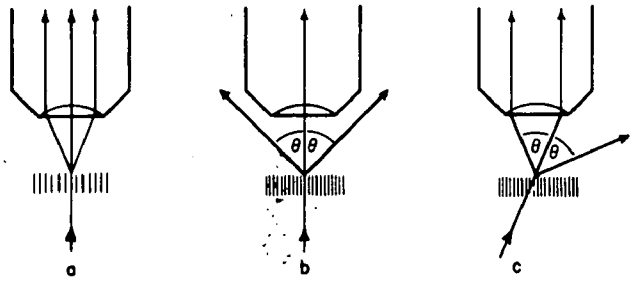


Figure 16

ABBE THEORY OF RESOLUTION

b The condenser should be well-corrected and have a numerical aperture as high as the objective to be used.

c An apochromatic oil-immersion objective should be used with a compensating eyepiece of at least 15X magnification. The immersion oil should have an index close to 1.515 and have proper dispersion for the objective being used.

d Immersion oil should be placed between the condenser and slide and between cover slip and objective. The preparation itself should be surrounded by a liquid having a refractive index of 1.515 or more.

e The illumination should be reasonably monochromatic and as short in wavelength as possible. An interference filter transmitting a wavelength of about 480-500 millimicrons is a suitable answer to this problem. Ideally, of course, ultraviolet light should be used to decrease the wavelength still further.

The practical effect of many of these factors is critically discussed by Loveland[2] in a paper on the optics of object space.

IV PHOTOMICROGRAPHY

A Introduction

Photomicrography, as distinct from microphotography, is the art of taking pictures through the microscope. A microphotograph is a small photograph, a photomicrograph is a photograph of a small object. Photomicrography is a valuable tool in recording the results of microscopical study. It enables the microscopist to

1 describe a microscopic field objectively without resorting to written descriptions,

2 record a particular field for future reference,

3 make particle size counts and counting analyses easily and without tying up a microscope,

4 enhance or exaggerate the visual microscopic field to bring out or emphasize certain details not readily apparent visually,

5 record images in ultraviolet and infrared microscopy which are otherwise invisible to the unaided eye.

There are two general approaches to photomicrography, one requires only a plate or film holder supported above the eyepiece of the microscope with a light-tight bellows, the other utilizes any ordinary camera with its own lens system, supported with a light-tight adaptor above the eyepiece. It is best, in the latter case, to use a reflex camera so that the image can be carefully focused on the ground glass. Photomicrography of this type can be regarded simply as replacing the eye with the camera lens system. The camera should be focused at infinity, just as the eye is for visual observation, and it should be positioned close to and over the eyepiece.

The requirements for photomicrography, however, are more rigorous than those for visual work. The eye can normally compensate for varying light intensities, curvature of field and depth of field. The photographic plate, however, lies in one plane, hence the greatest care must be used to focus sharply on the subject plane of interest and to select optics to give minimum amounts of field curvature and chromatic aberrations.

With black and white film, color filters may be used to enhance the contrast of some portions of the specimen while minimizing chromatic aberrations of the lenses. In color work, however, filters cannot usually be used for this purpose and better optics may be required.

Photomicrographic cameras which fit directly onto the microscope are available in 35-mm or up to 3-1/4 × 4-1/4 inch sizes. Others are made which accommodate larger film sizes and which have their own support independent of the microscope. The former, however, are preferred for ease of handling and lower cost. The latter system is preferred for greater flexibility and versatility and lack of vibration. The Polaroid camera has many applications in microscopy and can be used on the microscope directly but, because of its weight, only when the microscope has a vertically moving stage for focusing rather than a focusing body tube.

B Determination of Correct Exposure

Correct exposure determination can be accomplished by trial and error, by relating new conditions to previously used successful conditions and by photometry.

With the trial and error method a series of trial exposures is made, noting the type of subject, illumination, filters, objective, eyepiece, magnification, film and shutter speed. The best exposure is selected. The following parameters can be changed and the exposure time adjusted accordingly:

1 Magnification. Exposure time varies as the square of the magnification.

Example Good exposure was obtained with a 1/10-second exposure and a magnification of 100X. If the magnification is now

200X, the correct exposure is calculated as follows:

new exposure time = old exposure time

$\times \left(\frac{\text{new magnification}}{\text{old magnification}}\right)^2 = 1/10 \left(\frac{200}{100}\right)^2 =$

4/10 or, say, 1/2 second.

It should be noted, however, that the above calculation can be made only when there has been no change in the illumination system including the condenser or the objective. Only changes in magnification due to changing eyepieces or bellows extension distance can be handled in the above manner.

2 Numerical aperture. Exposure time varies inversely as the square of the smallest working numerical aperture of the condenser and objective.

Example: Good exposure was obtained at 1/10 second with the 10X objective, N.A. 0.25, at full aperture. With a 20X objective, N.A. 0.25, at full aperture and the same final magnification, what is the correct exposure time?

new exposure time = old exposure time

$\times \left(\frac{\text{old N.A.}}{\text{new N.A.}}\right)^2 = 1/10 \left(\frac{0.25}{0.50}\right)^2 = 1/40$ or, say, 1/50 second.

It is seen that more light reaches the photographic film with higher numerical apertures at the same magnification.

3 Film. Exposure time varies inversely with the American Standards Association speed index of the film.

Example: A good picture was obtained with Eastman Tri-X film at 1/100 second. What is the correct exposure for Eastman Kodachrome II Type A. The A.S.A. speed for Tri-X is 400 and for Kodachrome II Type A Professional is 40.

new exposure time = old exposure time

$\times \frac{\text{A. S. A. of old film}}{\text{A. S. A. of new film}} = 1/100(400/40) =$

10/100 or 1/10 second.

4 Other parameters may be varied but the prediction of exposure time cannot be made readily. Experience and photoelectric devices are the best guides to the proper exposure.

Photoelectric devices are excellent for determining correct exposure. Since ordinary photographic exposure meters are not sensitive enough for photomicrography, more sensitive instruments, having a galvanometer or electronic amplifying circuit, are required. Some photosensitive cells are inserted in the body tube in place of the eyepiece for light intensity readings. This has the advantage of detecting the light level at a point of high intensity but does not take into account the eyepiece, the distance to the film or the film speed.

The cell may be placed just above the eyepiece so that it registers the total amount of light leaving the eyepiece. Again, the effects of film speed and the projection distance are not accounted for. The principal drawback with the total light measuring photometer is the difficulty of taking into account the area of field covered. Take, for example, a bright field in which only a few crystals appear, perhaps 1 percent of the light entering the field of view is scattered by the crystals and the photometer shows close to a maximum reading. Now assume that everything remains constant except the number of crystals and, consequently, the amount of light scattered. The photometer reading could easily drop by 50 percent, yet the proper exposure is unchanged. The situation is similar for photomicrography with crossed polars since the photometer reading depends on the intensity of illumination, on the birefringence and thickness of the crystals and

on the number and size of the crystals in the field or, alternatively, on the area of the field covered by birefringent crystals. One of the best solutions to this problem is to measure the photometer reading with no preparation on the stage. A first-order red compensator or a quartz wedge is inserted when crossed polars are being used to illuminate the entire field.

An alternative is to place the cell on the ground glass where the film will be located. However, although all variables except film speed are now taken into account, measurements in the image plane have the disadvantage of requiring a more sensitive electronic photoelectric apparatus.

No matter what method is used for placing the photocell, the exposure time can be determined by the general formula

$$\text{exposure time} = \frac{k}{\text{meter reading}}.$$

The constant k will depend on the physical arrangement and film used. To determine k for any particular system, first set up the microscope to take a picture. Record the meter reading and take a series of trial exposures. Pick out the best exposure and calculate k. Then the k which was determined holds as long as no change is made in the light path beyond the photocell, e.g. changing to a faster film or changing the projection distance. Thus the objective, condenser position or illuminator may be changed without affecting k if the cell is used as described above.

Example: With one particular arrangement of photocell and film, the meter reading is found to be 40. A series of photographs are taken at 1/2, 1/5, 1/10, 1/25 and 1/50 seconds. The photomicrograph taken at 1/5 second is judged to be the best; hence k is calculated as follows:

k = meter reading × exposure time = 40 × 1/5 = 8.

Assume now that a new picture is to be taken at another magnification (but with the same film and projection distance) and that the new meter reading is 16, therefore

exposure time = k/meter reading = 8/16 = 1/2 second.

V MICROMETRY

A Particle Size Determination

Linear distances and areas can be measured with the microscope. This permits determination of particle size and quantitative analysis of physical mixtures. The usual unit of length for microscopical measurements is the micron (1×10^{-3}mm or about 4×10^{-5}inch). Measuring particles in electron microscopy requires an even smaller unit, the millimicron (1×10^{-3} micron or 10 Angstrom units). Table 6 shows the approximate average size of a few common airborne materials.

Table 6. APPROXIMATE PARTICLE SIZE OF SEVERAL COMMON PARTICULATES

Ragweed pollen	25 microns
Fog droplets	20 microns
Power plant flyash (after precipitators)	2-5 microns
Tobacco smoke	0.2 micron (200 millimicrons)
Foundry fumes	0.1 - 1 micron (100-1000 millimicrons)

The practical lower limit of accurate particle size measurement with the light microscope is about 0.5 micron. The measurement of a particle smaller than this with the light microscope leads to errors which, under the best circumstances, increase to about ± 100 percent (usually +).

One of the principal uses of high resolving power is in the precise measurement of

particle size. There are, however, a variety of approximate and useful procedures as well.

1 Methods of particle size measurement

a Knowing the magnification of the microscope (product of the magnification of objective and eyepiece), the size of particles can be estimated. For example, with a 10X eyepiece and a 16-mm (or 10X) objective, the total magnification is 100X. A particle that appears to be 10-mm at 10 inches from the eye has an actual size of 10 mm divided by 100 or 0.10 mm or 100 microns. This is in no sense an accurate method, but it does permit quick estimation of particle size, the error in this estimation is usually 10-25 percent.

b Another approximate method is also based on the use of known data. If we know approximately the diameter of the microscope field, we can estimate the percentage of the diameter occupied by the object to be measured and calculate from these figures the approximate size of the object. The size of the microscope field depends on both the objective and the ocular although the latter is a minor influence. The size of the field should be determined with a millimeter scale for each objective and ocular. If this is done, estimation of sizes by comparison with the entire field diameter can be quite accurate (5-10%).

c The movement of a graduated mechanical stage can also be used for rough measurement of diameters of large particles. Stages are usually graduated (with vernier) to read to 0.1 millimeter, or 100 microns. In practice, the leading edge of the particle is brought to one of the lines of the cross hair in the eyepiece and a reading is taken of the stage position. Then the particle is moved across the field by moving the mechanical stage in an appropriate direction until the second trailing edge just touches the cross-hair line. A second reading is taken and the difference in the two readings is the distance moved or the size of the particle. This method is especially useful when the particle is larger than the field, or when the optics give a distorted image near the edge of the field.

d The above method can be extended to projection or photography. The image of the particles can be projected on a screen with a suitable light source or they may be photographed. The final magnification, M, on the projection surface (or film plane) is given approximately by

$$M = D \times O.M. \times E.M./25$$

where O.M. = objective magnification
E.M. = eyepiece magnification
D = projection distance from the eyepiece in centimeters.

The image detail can then be measured in centimeters and the actual size computed by dividing by M. This method is usually accurate to within 2-5 percent depending on the size range of the detail measured.

e The stated magnifications and/or focal lengths of the microscope optics are nominal and vary a bit from objective to objective or eyepiece to eyepiece. To obtain accurate measurements, a stage micrometer is used to calibrate each combination of eyepiece and objective. The stage micrometer is a glass microscope slide that has, accurately engraved in the center, a scale, usually 2 millimeters long, divided into 200 parts, each part representing 0.01 millimeter. Thus when this scale is observed, projected or photographed, the exact image magnification can be determined. For example, if 5 spaces of the stage micrometer measure 6 millimeters when projected, the actual magnification is

$$\frac{6}{5\ (0.01)} = 120 \text{ times.}$$

This magnification figure can be used to improve the accuracy of method 4 above.

f The simplest procedure and the most accurate is based on the use of a micrometer eyepiece. Since the eyepiece magnifies a real image from the objective, it is possible to place a transparent scale in the same plane as the image from the objective and thus have a scale superimposed over the image. This is done by first placing an eyepiece micrometer scale disc in the eyepiece. The eyepiece micrometer has an arbitrary scale and must be calibrated with each objective used. The simplest way to do this is to place the stage micrometer on the stage and note a convenient whole number of eyepiece micrometer divisions. The value in microns for each eyepiece micrometer division is then easily computed. When the stage micrometer is removed and replaced by the specimen, the superimposed eyepiece scale can be used for accurate measurement of any feature in the specimen by direct observation, photography or projection.

2 Calibration of eyepiece micrometer

Each micrometer stage scale has divisions 100μ (0.1 mm) apart; one or two of these are usually subdivided into 10μ (0.01-mm) divisions. These form the standard against which the arbitrary divisions in the micrometer eyepiece are to be calibrated. Each objective must be calibrated separately by noting the correspondence between the stage scale and the eyepiece scale. Starting with the lowest power objective focus on the stage scale, arrange the two scales parallel and in good focus. It should be possible to determine the number of eyepiece divisions exactly equal to some whole number of divisions of the stage scale, a distance readily expressed in microns.

The calibration consists, then, of calculating the number of microns per eyepiece scale division. To make the comparison as accurate as possible, a large part of each scale must be used (see Figure 17). Let's assume that with the low power 16-mm objective 6 large divisions of the stage scale (s.m.d.) are equal to 38 divisions of the eyepiece scale. This means that 38 eyepiece micrometer divisions (e.m.d.) are equivalent to 600 microns. Hence:

$$1 \text{ e.m.d.} = 600/38 = 15.8\mu.$$

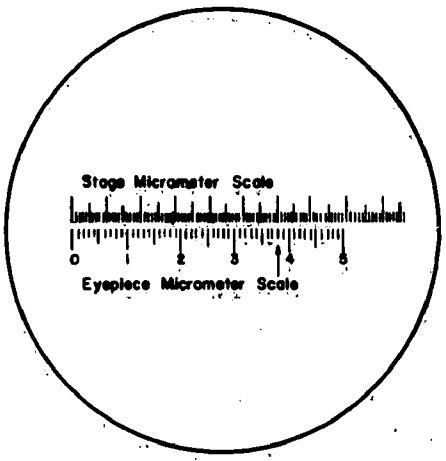


Figure 17

COMPARISON OF STAGE MICROMETER SCALE WITH EYEPIECE MICROMETER SCALE

Thus when that micrometer eyepiece is used with that 16-mm objective each division of the eyepiece scale is equivalent to 15.8μ, and it can be used to make an accurate measurement of any object on the microscope stage. A particle, for example, observed with the 16-mm objective and measuring 8.5 divisions on the eyepiece scale is 8.5 (15.8) or 135μ in diameter.

Each objective on your microscope must be calibrated in this manner.

A convenient way to record the necessary data and to calculate μ/emd is by means of a table.

Table 7

Objective	No. smd = no. emd	μ = no. emd	μ = 1 emd
32-mm	18 = 44	1800 = 44	40.9μ
16-mm	6 = 38	600 = 38	15.8μ
4-mm	1 = 30	100 = 30	3.33μ

3 Determination of particle size distribution

The measurement of particle size can vary in complexity depending on particle shape. The size of a sphere may be denoted by its diameter. The size of a cube may be expressed by the length of an edge or diagonal. Beyond these two configurations, the particle "size" must include information about the shape of the particle in question, and the expression of this shape takes a more complicated form.

Martin's diameter is the simplest means of measuring and expressing the diameters of irregular particles and is sufficiently accurate when averaged for a large number of particles. In this method, the horizontal or east-west dimension of each particle which divides the projected area into halves is taken as Martin's diameter (Figure 18).

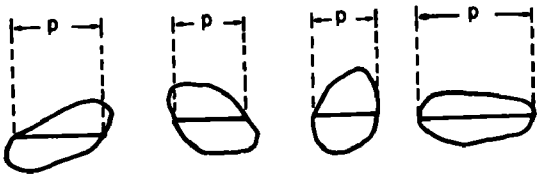


Figure 18

MARTIN'S DIAMETER

The more particles counted, the more accurate will be the average particle size. Platelike and needlelike particles should have a correction factor applied to account for the third dimension since all such particles are restricted in their orientation on the microscope slide. When particle size is reported, the general shape of the particles as well as the method used to determine the "diameter" should be noted.

Particle size distribution is determined routinely by moving a preparation of particles past an eyepiece micrometer scale in such a way that their Martin's diameter can be tallied. All particles whose centers fall within two fixed divisions on the scale are tallied. Movement of the preparation is usually accomplished by means of a mechanical stage but may be carried out by rotation of an off-center rotating stage. A sample tabulation appears in Table 8. The eyepiece and objective are chosen so that at least six, but not more than twelve, size classes are required and sufficient particles are counted to give a smooth curve. The actual number tallied (200 - 2,000) depends on particle shape regularity and the range of sizes. The size tallied for each particle is that number of eyepiece micrometer divisions most closely approximating Martin's diameter for that particle.

4 Calculation of size averages

The size data may be treated in a variety of ways, one simple, straightforward treatment is shown in Table 9. For a more complete discussion of the treatment of particle size data see Chamot and Mason's Handbook of Chemical Microscopy[3], page 26.

The averages with respect to number, $\bar{d}_1$, surface, $\bar{d}_3$, and weight or volume, $\bar{d}_4$, are calculated as follows for the data in Table 9.

Table 8. PARTICLE SIZE TALLY FOR A SAMPLE OF STARCH GRAINS

Size class (emd*)	Number of particles	Total
1	𝍸 𝍸 𝍸 1	16
2	𝍸 𝍸 𝍸

Table 9. CALCULATIONS FOR PARTICLE SIZE AVERAGE

d (Aver. diam. in emd)	n	nd	nd^2	nd^3	nd^4
1	16	16	16	16	16
2	98	196	392	784	1568
3	110	330	990	2970	8910
4	107	428	1712	6848	27392
5	71	355	1775	8875	44375
6	45	270	1620	9720	58320
7	21	147	1029	7203	50421
8	2	16	128	1024	8192
	470	1758	7662	37440	199194

B Counting Analysis

Mixtures of particulates can often be quantitatively analyzed by counting the total number of particulates from each component in a representative sample. The calculations are, however, complicated by three factors: average particle size, particle shape and the density of the components. If all of the components were equivalent in particle size, shape and density then the weight percentage would be identical to the number percentage. Usually, however, it is necessary to determine correction factors to account for the differences.

When properly applied, this method can be accurate to within $\pm$ 1 percent and, in special cases, even better. It is often applied to the analysis of fiber mixtures and is then usually called a dot-count because the tally of fibers is kept as the preparation is moved past a point or dot in the eyepiece.

A variety of methods can be used to simplify recognition of the different components. These include chemical stains or dyes and enhancement of optical differences such as refractive indices, dispersion or color. Often, however, one relies on the differences in morphology, e.g. counting the percent of rayon fibers in a sample of "silk".

Example 1: A dot-count of a mixture of fiberglass and nylon shows

nylon	262
fiberglass	168

Therefore

$$\% \text{ nylon} = 262/(262 + 168) \times 100 = 60.9\% \text{ by number.}$$

However, although both fibers are smooth cylinders, they do have different densities and usually different diameters. To correct for diameter one must measure the average diameter of each type of fiber and calculate the volume of a unit length of each.

	aver. diam. μ	volume of 1-μ slice, μ^3
nylon	18.5	268
fiberglass	13.2	117

The percent by volume is, then:

$$\% \text{ nylon} = \frac{262 \times 268}{(262 \times 268)+(168 \times 117)} \times 100$$

$$= 78.1\% \text{ by volume.}$$

Still we must take into account the density of each in order to calculate the weight percent.

If the densities are 1.6 for nylon and 2.2 for glass then the percent by weight is

$$\% \text{ nylon} = \frac{262 \times 268 \times 1.6}{(262 \times 268 \times 1.6)+(168 \times 117 \times 2.2)} \times 100$$

$$= 72\% \text{ by weight.}$$

Example 2 A count of quartz and gypsum shows

quartz	283
gypsum	467

To calculate the percent by weight we must take into account the average particle size, the shape and the density of each.

The average particle size with respect to weight, $\bar{d}_4$, must be measured for each and the shape factor must be determined. Since gypsum is more platelike than quartz each particle of gypsum is thinner. The shape factor can be approximated or can be roughly calculated by measuring the actual thickness of a number of particles. We might find, for example, that gypsum particles average 80% of the volume of the average quartz particle, this is our shape factor. The final equation for the weight percent is:

$$\% \text{ quartz} = \frac{283 \times \pi \bar{d}_4/6 \times Dq}{238 \times \pi \bar{d}_4/6 \times Dq + 467 \times \pi \bar{d}_4^*/6 \times 0.80 \times Dg} \times 100$$

where Dq and Dg are the densities of quartz and gypsum respectively, 0.80 is the shape factor and $\bar{d}_4$ and $\bar{d}_4^*$ are the average particle sizes with respect to weight for quartz and gypsum respectively.

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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)

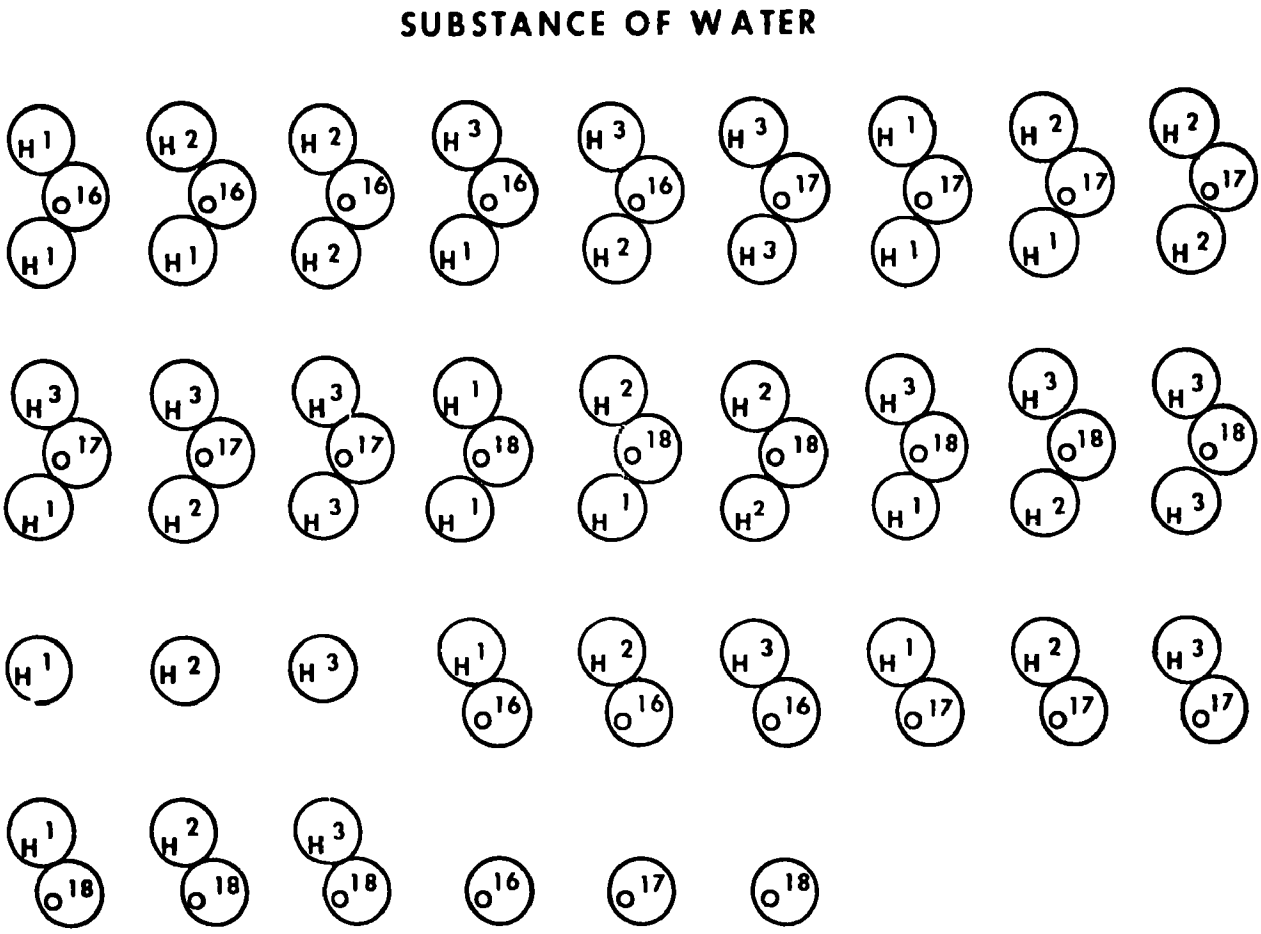

Figure 1

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	.99997	
8	.99988	
10	0.00973	

* 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_{18} 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.

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 been reported from 34 to 160 atm. This 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 arhythmic water movements which have had major study only in oceanography. 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.

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.

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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 (Odum, 1959).

1 From a functional standpoint, an ecosystem has two component parts. (Figures 1, 2, 3)

a Autotrophic (self-nourishing) organisms are able to fix light energy and manufacture food from simple inorganic substances.

b Heterotrophic (other-nourishing) organisms utilize, rearrange, and decompose the complex materials synthesized by the autotrophs.

2 From a structural standpoint, it is convenient to recognize four constituents as comprising an ecosystem.

a Abiotic substances are basic or essential mineral elements and compounds.

b Autotrophic (holophytic) organisms are the producers, largely the green plants.

c Heterotrophic (or holozoic) organisms are chiefly animals that ingest or consume other organisms or particulate organic matter.

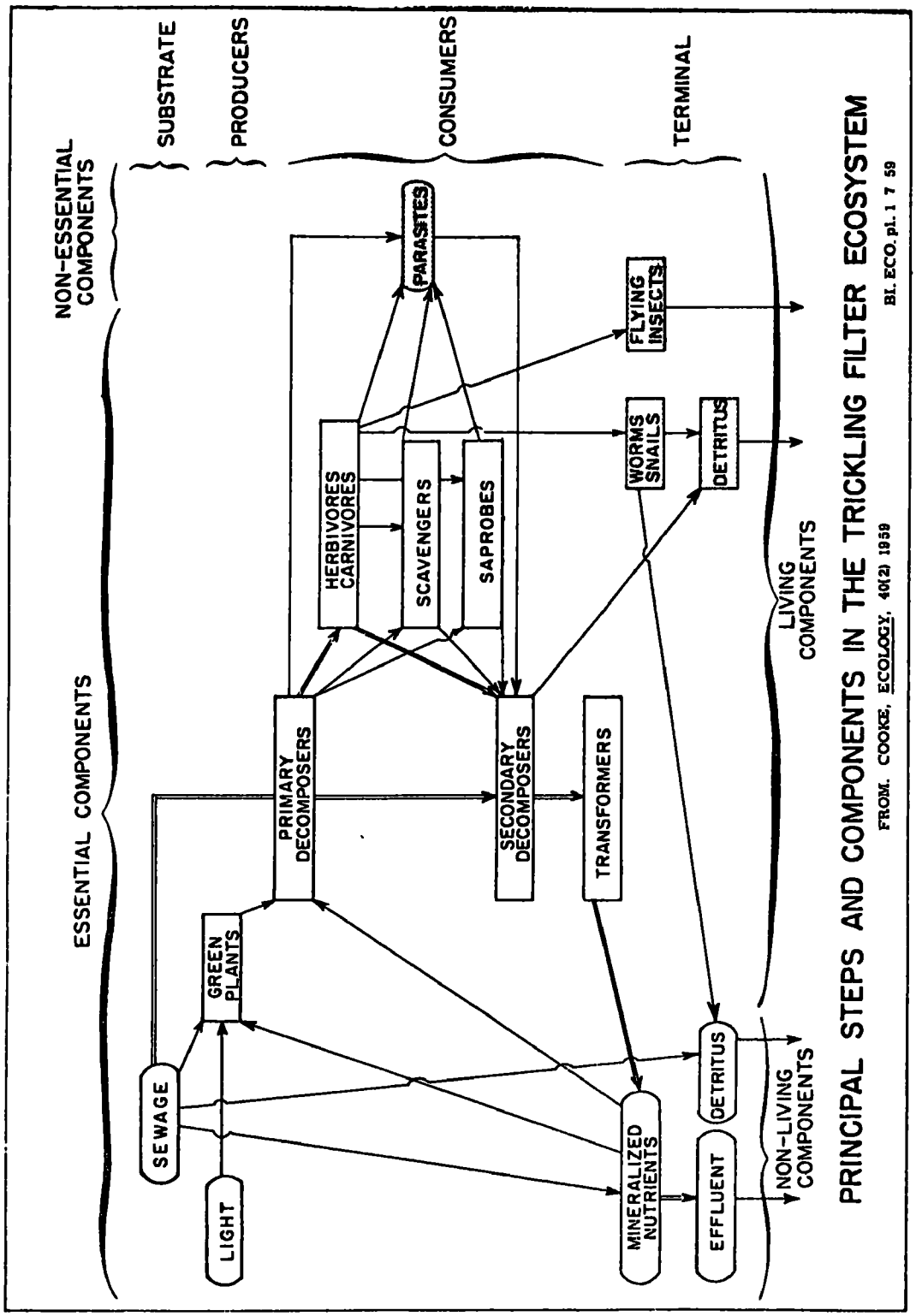
NON-ESSENTIAL COMPONENTS
ESSENTIAL COMPONENTS
SUBSTRATE
PRODUCERS
CONSUMERS
TERMINAL
SEWAGE
LIGHT
GREEN PLANTS
PRIMARY DECOMPOSERS
SECONDARY DECOMPOSERS
TRANSFORMERS
HERBIVORES CARNIVORES
SCAVENGERS
SAPROBES
PARASITES
FLYING INSECTS
WORMS SNAILS
DETRITUS
MINERALIZED NUTRIENTS
EFFLUENT
DETRITUS
LIVING COMPONENTS
NON-LIVING COMPONENTS
PRINCIPAL STEPS AND COMPONENTS IN THE TRICKLING FILTER ECOSYSTEM
FROM. COOKE, ECOLOGY, 40(2) 1959
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Figure 1

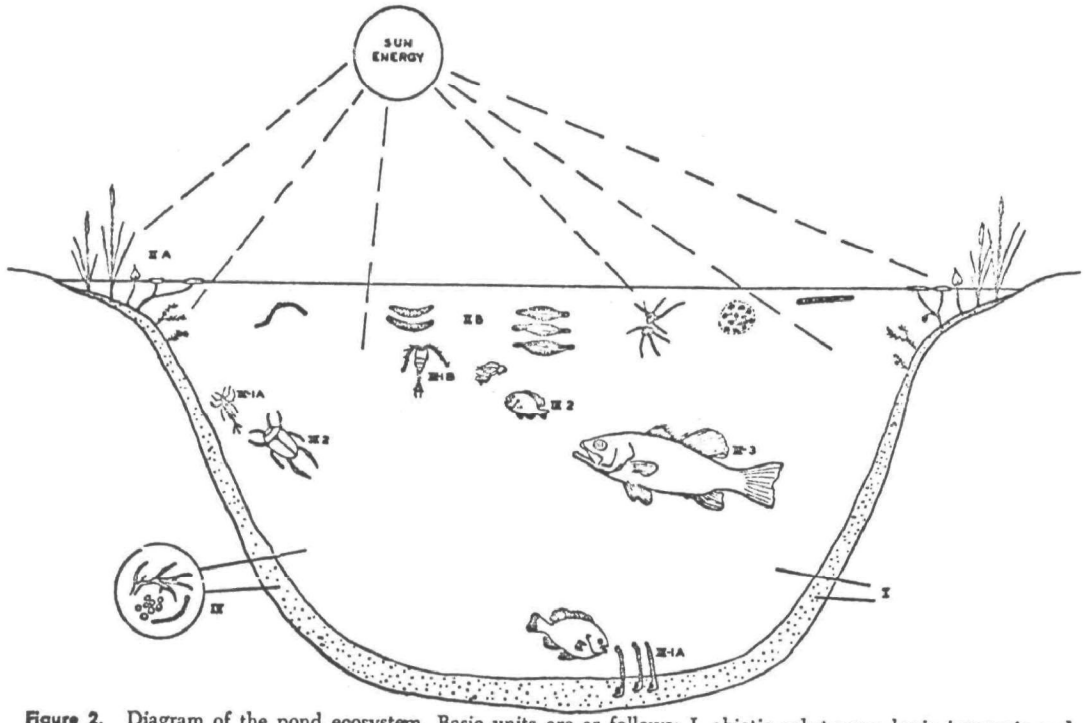


Figure 2. Diagram of the pond ecosystem. Basic units are as follows: I, abiotic substances–basic inorganic and organic compounds; IIA, producers–rooted vegetation; IIB, producers–phytoplankton; III-1A, primary consumers (herbivores)–bottom forms; III-1B, primary consumers (herbivores)–zooplankton; III-2,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

d Decomposers are heterotrophic organisms, chiefly bacteria and fungi that return or reduce the complex compounds of dead protoplasm to their original mineral condition.

B Functioning of the Ecosystem

1 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.

2 A food web is the interlocking pattern of food chains in an ecosystem. (Figure 1) 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.

3 Trophic levels

a First - Green plants (producers) (Figure 4)fix biochemical energy and synthesize basic organic substances.

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

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

d Fourth - Secondary carnivores feed on primary carnivores.

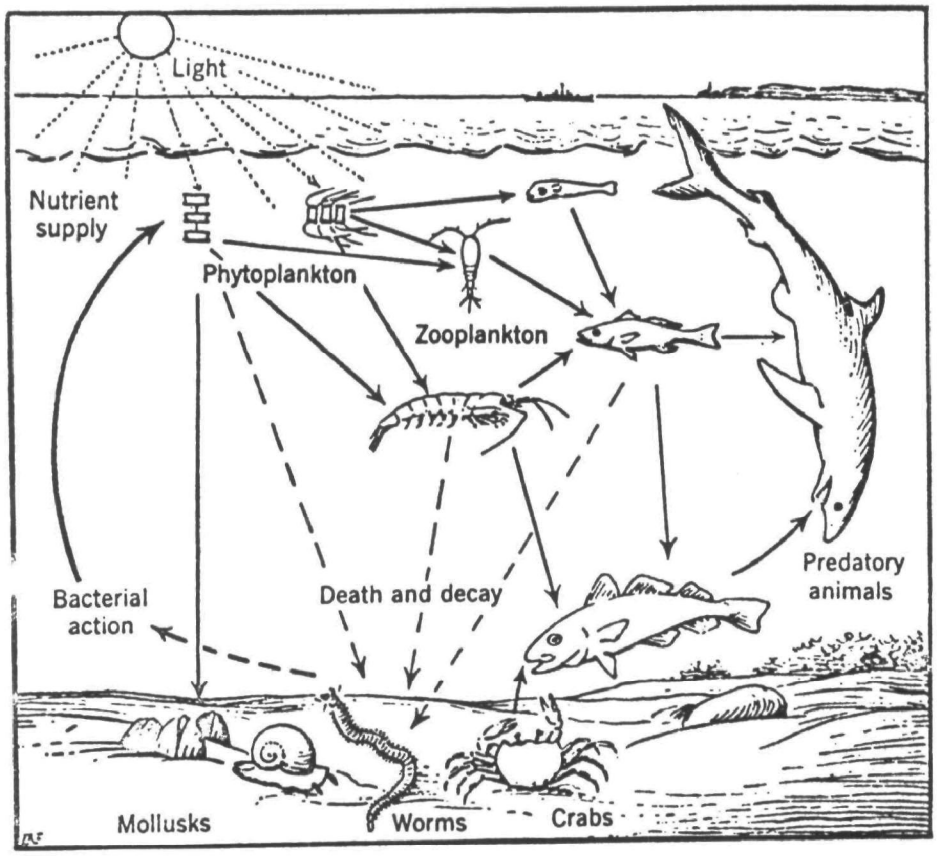
e Last - Ultimate carnivores are the last or ultimate level of consumers.

4 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.

5 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



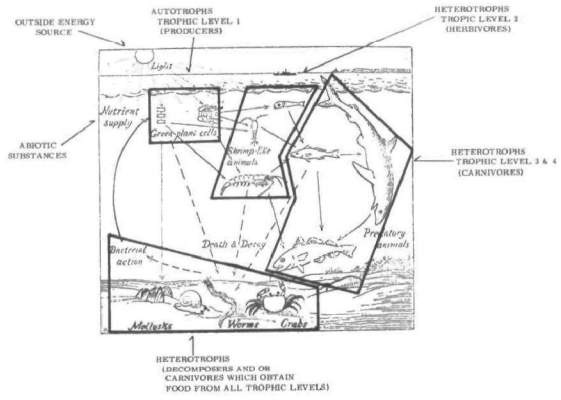


Figure 3. A MARINE ECOSYSTEM (After Clark, 1954 and Patten, 1966)

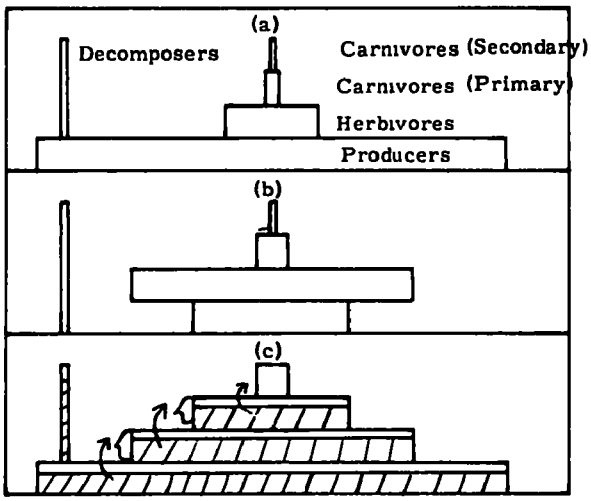


Figure 4. HYPOTHETICAL PYRAMIDS of (a) Numbers of individuals, (b) Biomass, and (c) Energy (Shading Indicates Energy Loss).

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 4).

IV 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.

V 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.

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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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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

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)

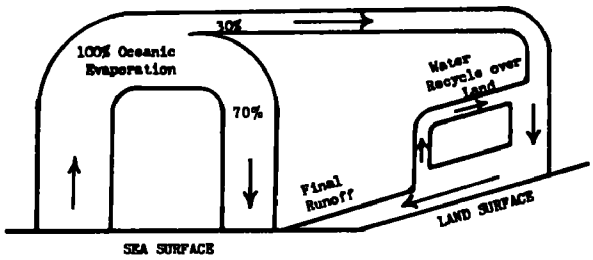


Figure 1. THE WATER CYCLE

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

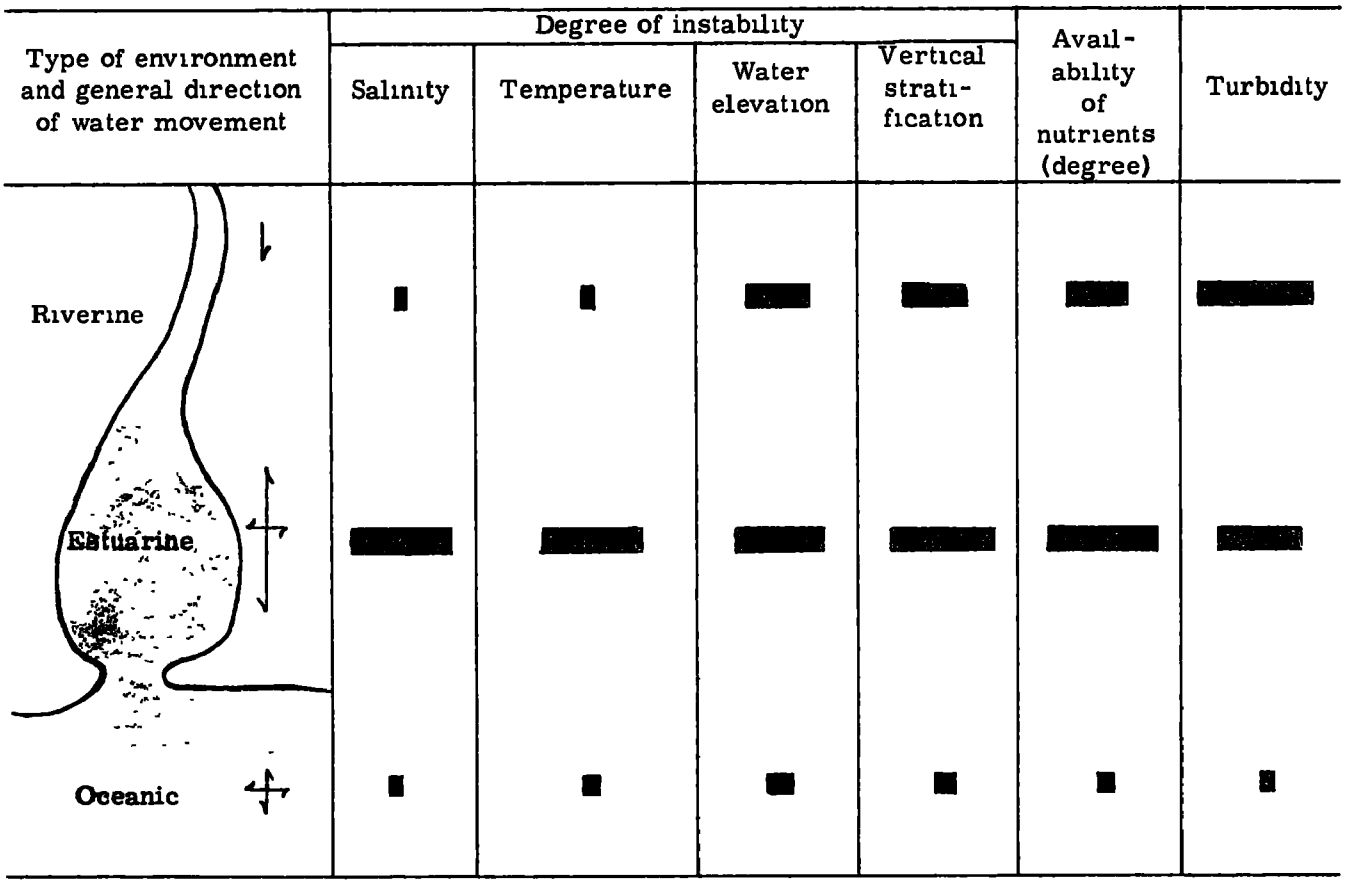


Figure 2. RELATIVE VALUES OF VARIOUS PHYSICAL AND CHEMICAL FACTORS FOR RIVER, ESTUARINE, AND OCEANIC ENVIRONMENTS

B Biotic Factors

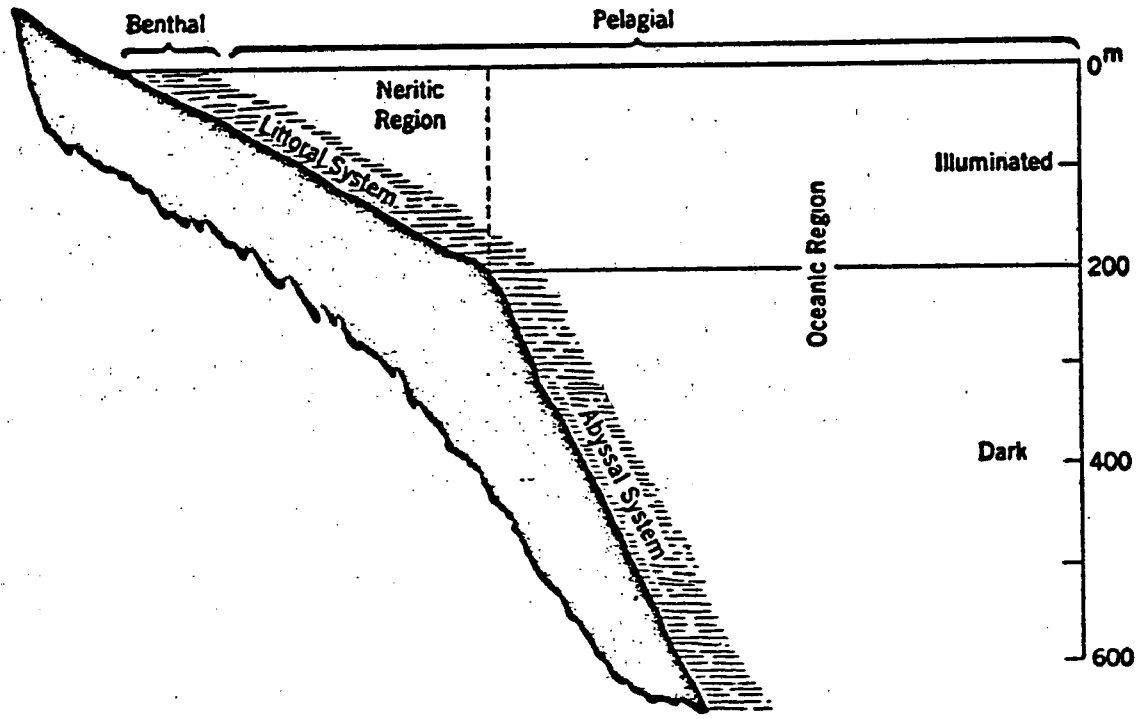
1 A complex of physical and chemical factors determine the biotic composition of an environment. In general, the number of species in a highly variable environment tends to be less than the number in a more stable environment (Hedgpeth, 1966).

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.

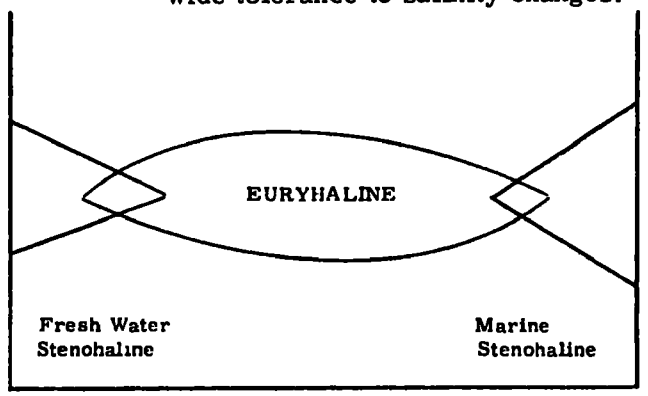


Figure 4. Salinity Tolerance of Organisms

b Eurythermal - wide tolerance to temperature changes.

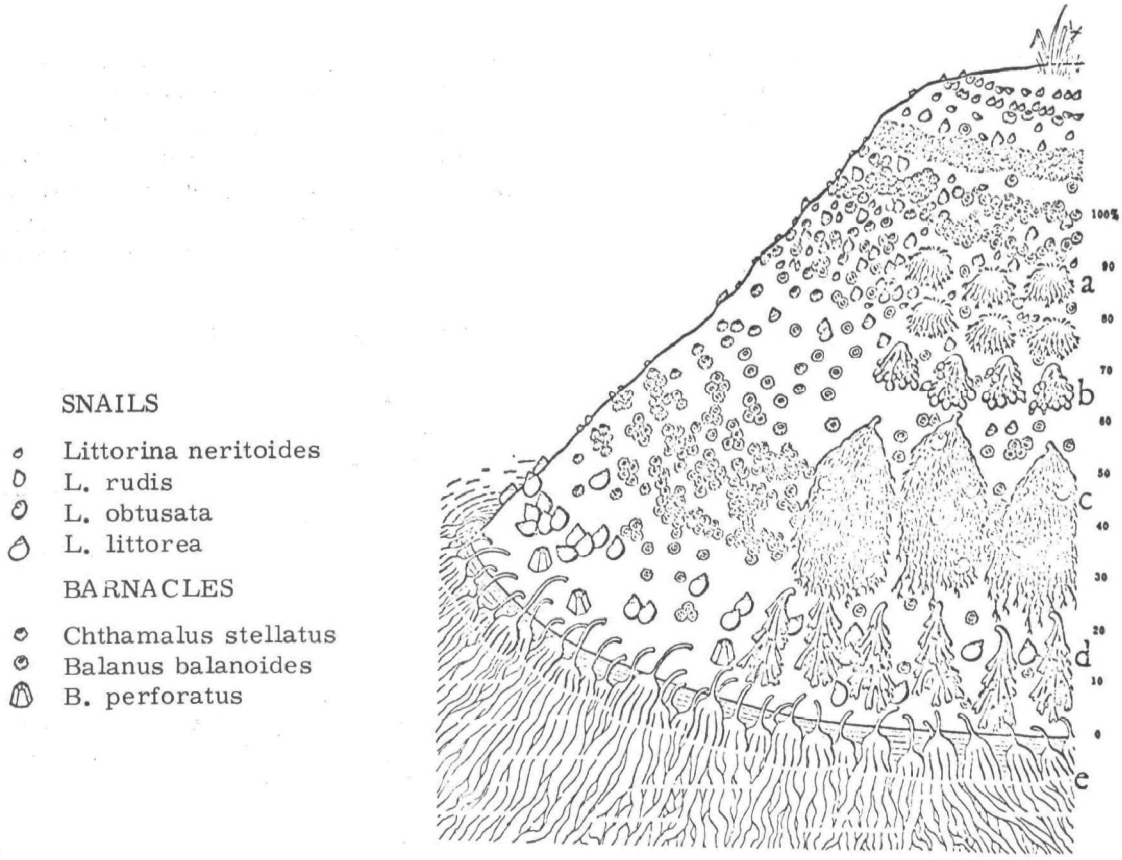


Figure 5

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 Stenothernal - 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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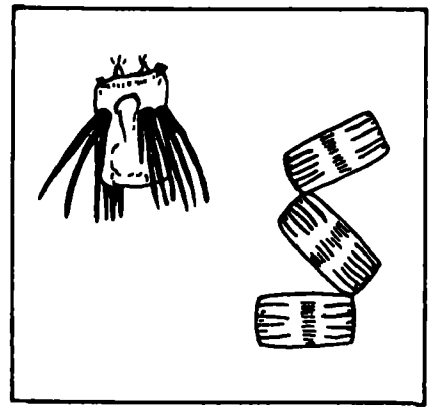
This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.



CHAPTER II

IDENTIFICATION OF PLANKTON AND ASSOCIATED ORGANISMS

Structure and Function of Cells 1

Aquatic Organisms of Significance in Plankton Surveys 2

Types of Algae 3

Blue-Green Algae 4

Green and Other Pigmented Flagellates 5

Filamentous Green Algae 6

Coccoid Green Algae 7

Diatoms 8

Filamentous Bacteria 9

Protozoa, Nematodes, and Rotifers 10

Free-Living Amoebae and Nematodes 11

Animal Plankton 12

STRUCTURE AND FUNCTION OF CELLS

I INTRODUCTION

What are cells? Cells may be defined as the basic structural units of life. The cell has many different parts which carry on the various functions of cell life. These are called organelles ("little organs").

A The branch of biology which deals with the form and structure of plants and animals is called "Morphology." The study of the arrangement of their several parts is called "anatomy", and the study of cells is called "cytology".

B There is no "typical" cell, for cells differ from each other in detail, and these differences are in part responsible for the variety of life that exists on the earth.

II FUNDAMENTALS OF CELL STRUCTURE

A How do we recognize a structure as a cell? We must look for certain characteristics and/or structures which have been found to occur in cells. The cell is composed of a variety of substances and structures, some of which result from cellular activities. These include both living and non-living materials.

1 Non-living components include:

a A "cell wall" composed of cellulose may be found as the outermost covering of many plant cells.

b "Vacuoles" are chambers in the protoplasm which contain fluids of different densities (i.e., different from the surrounding protoplasm).

2 The "living" parts of the cell are called "protoplasm." The following structures are included:

a A thin "cell membrane" is located just inside the cell wall. This membrane may be thought of as the outermost layer of protoplasm.

b In plant cells the most conspicuous protoplasmic structures are the "chloroplasts", which contain highly organized membrane systems bearing the photosynthetic pigments (chlorophylls, carotenoids, and xanthophylls) and enzymes.

c The "nucleus" is a spherical body which regulates cell function by controlling enzyme synthesis.

d "Granules" are structures of small size and may be "living" or non-living" material.

e "Flagella" are whip-like structures found in both plant and animal cells. The flagella are used for locomotion, or to circulate the surrounding medium.

f "Cilia" resemble short flagella, found almost exclusively on animal cells. In the lower animals, cilia are used for locomotion and food gathering.

g The "pseudopod", or false foot, is an extension of the protoplasm of certain protozoa, in which the colloidal state of the protoplasm alternates from a "sol" to a "jel" condition from time to time to facilitate cell movement.

h "Ribosomes" are protoplasmic bodies which are the site of protein synthesis. They are too small (150 Å in diameter) to be seen with a light microscope.

i "Mitochondria" are small membranous structures containing enzymes that oxidize food to produce energy transfer compounds (ATP).

B How basic structure is expressed in some major types of organisms.

We can better visualize the variety of cell structure by considering several specific cells.

1 Bacteria have few organelles, and are so minute that under the light microscope only general morphological types (i.e., the three basic shapes; rods, spheres, and spirals) can be recognized. The following structures have been defined:

a The "capsule" is a thick protective covering of the cell exterior, consisting of polysaccharide or polypeptide.

b The cell wall and plasma membrane are present.

c Although no well defined nucleus is visible in bacterial cells, the electron microscope has revealed areas of deoxyribose nucleic acid (DNA) concentration. This substance is present within the nucleus of of higher cells, and is the genetic or hereditary material.

d Some types of bacteria contain a special type of chlorophyll (bacteriochlorophyll) and carry on photosynthesis.

2 The blue-green algae are similar to the bacteria in structure, but contain the photosynthetic pigment chlorophyll a.

a Like the bacteria, these forms also lack an organized nucleus (the nuclear region is not bounded by a membrane).

b The chlorophyll-bearing membranes are not localized in distinct bodies (chloroplasts), but are dispersed throughout the cell.

c Gas-filled structures called "pseudovacuoles" are found in some types of blue-greens.

3 The green algae as a group include a great variety of structural types, ranging from single-celled non-motile forms to large motile colonies. Some types are large enough to resemble higher aquatic plants.

a The chloroplasts are modified into a variety of shapes and are located in different positions. Examples of chloroplast shape and position are:

1) Parietal - located on the periphery of the cell; usually cup-shaped and may extend completely around the inner surface of the plasma membrane.

2) Discoid - also located on the periphery of the cell, but are plate-shaped; usually many per cell.

3) Axial - lying in the central axis of the cell; may be ribbon-like or star-shaped.

4) Radial - have arms or processes that extend outward from the center of the cell (radiate), reaching the plasma membrane.

5) Reticulate - a mesh-like network that extends throughout volume of the cell.

b Located in the chloroplasts may be dense, proteinaceous, starch-forming bodies called "pyrenoids".

4 The flagellated algae possess one-to-eight flagella per cell. The chloroplasts may contain brown and/or red pigments in addition to chlorophyll.

a Reserve food may be stored as starch (Chlamydomonas) paramylon (Euglena), or as oil.

5 The protozoa are single-celled animals which exhibit a variety of cell structure.

a The amoebae move by means of pseudopodia, as described previously.

b The flagellated protozoa (Mastigophora) possess one or more flagella.

c The ciliates are the most highly modified protozoans. The cilia may be more or less evenly distributed over the entire surface of the cell, or may be localized.

III FUNCTIONS OF CELLS

What are the functions of cells and their structural components? Cellular function is called "life", and life is difficult to define. Life is characterized by processes commonly referred to as reproduction, growth, photosynthesis, etc.

A Microorganisms living in surface waters are subjected to constant fluctuations in the physical and chemical characteristic of the environment, and must constantly modify their activities.

1 The cell requires a cource of chemical energy to carry on life processes and successfully compete with other organisms. Plant cells may obtain this energy from light, which is absorbed by chlorophyll and converted into ATP or food reserves, or from the oxidation of food stuffs. Animal cells obtain energy only from the oxidation of food.

2 Cells must obtain raw materials from the environment in order to grow and carry out other life functions. Inorganic and organic materials may be taken up by passive diffusion or by "active transport". In the later process, energy is used to build up and maintain a higher concentration of a substance (such as phosphate) inside the cell than is found outside. Algae are able to synthesis organic matter from inorganic raw materials (carbon dioxide and water), with the aid of energy derived from light, whereas animal cells must obtain their organic matter "ready-made" by consuming other organisms, organic debris, or dissolved organics.

IV SUMMARY

The cell is made up of many highly specialized substructures. The types of substructures present, and their appearance (shape, color, etc.) are very important in understanding the role of the organism in the aquatic community, and in classification.

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This outline originally prepared by Michael E. Bender, Biologist, formerly with Training Activities, FWPCA, SEC. and revised by Cornelius I. Weber, March 1970.

AQUATIC ORGANISMS OF SIGNIFICANCE IN PLANKTON SURVEYS

I INTRODUCTION

A Any organism encountered in a survey is of significance. Out 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.

C Recognition implies identification and an understanding of general relationships. 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 been long 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 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:

1 Plante: 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 (as well as the higher and more complex types).

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

2 Within the Protista, two principle 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.

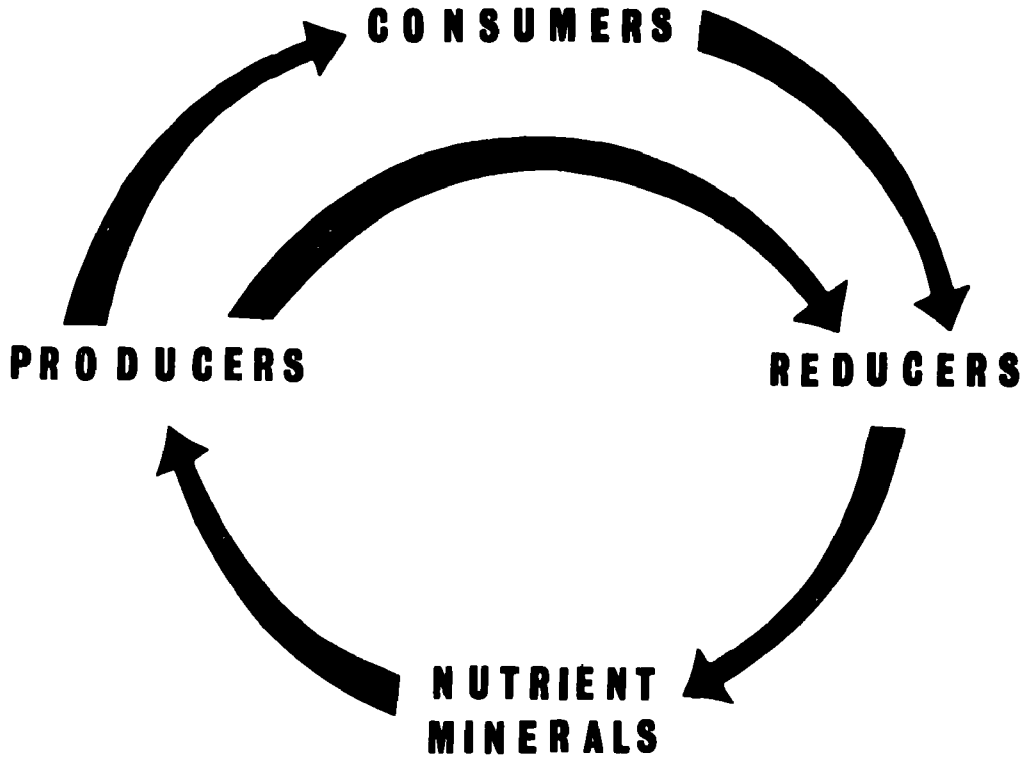
D Distributed throughout these groups will be found the traditional "phyla" of classic biology.

III PLANTS

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

1 Some types emerge above the surface.

2 Submersed types typically do not extend to the surface.



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" lack 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 freshwater 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 two pairs of 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) COPEPODS are marine and freshwater microcrustacea--swim by means of unbranched antennae.

4) OSTRACODS are the microscopic "clams with legs." Generally substrate oriented.

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 PROCHORDATES - 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 - snails, lizards and turtles

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

e AVES - birds - warm-blooded vertebrates with feathers

VI THE CLASSIFICATION OF ORGANISMS

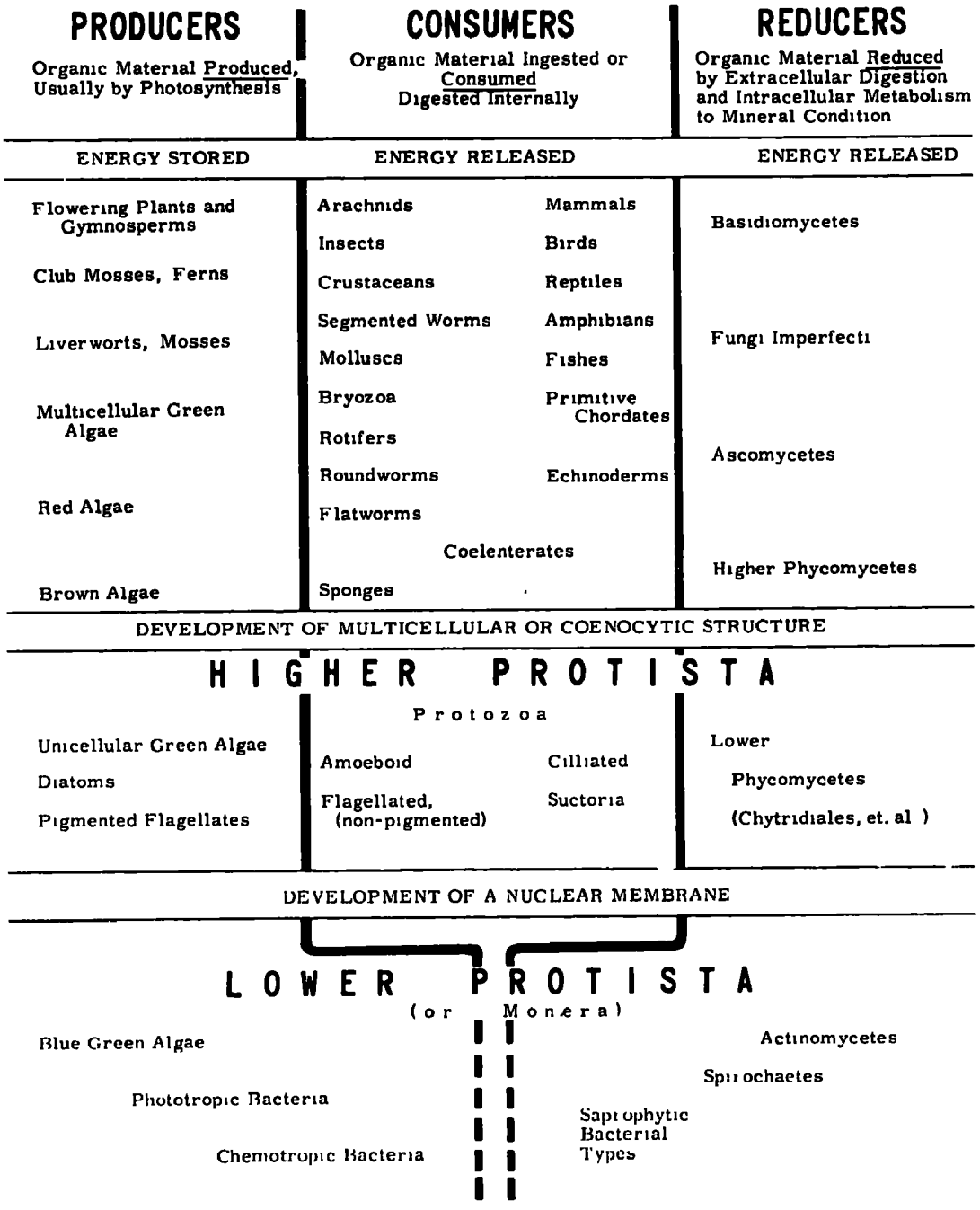
A INTRODUCTION

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.

B 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.

RELATIONSHIPS BETWEEN FREE LIVING AQUATIC ORGANISMS

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



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

2 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 subdivisions. 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).

3 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.

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

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

b The taxa (categories) employed are as follows:

The species is the foundation (plural: species)

Similar species are grouped into genera (singular: genus).

Similar genera are grouped into families.

Similar families are grouped into orders.

Similar orders are grouped into classes.

Similar classes are grouped into phyla (phylum).

Similar phyla are grouped into kingdoms.

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

5 The scientific name 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).

a 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

b 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.

6 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 Gomphonema olivaceum is:

Kingdom	Plantae
Phylum	Chrysophyta
Class	Bacillariophyceae
Order	Pennales
Family	Gomphonemaceae
Genus	Gomphonema
Species	olivaceum

a 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 sub-dividing 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.

b 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.

It should be emphasized that since most 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.

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.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center. Revised 1970 by R. M. Sinclair, Aquatic Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

TYPES OF ALGAE

I INTRODUCTION

A Algae - Simple plants with an autotrophic mode of nutrition. They contain chlorophyll, but lack the highly differentiated reproductive and body structures of higher plants. The algae range in size from less than one micron, to the giant kelp more than 300 ft. in length.

B Algae are carried about by water currents, wind, aquatic animals, water fowl, and insects, and are found everywhere. They grow actively at temperatures ranging from -2°C to 60°C.

C The algae play a more important role in aquatic habitats than on land. They serve as the basic energy source for other aquatic organisms, providing food for zoomicrobes, higher invertebrates and fish.

D There are two basic types of freshwater algal communities:

1 The phytoplankton - free floating algae

2 Phycoperiphyton - attached or bottom forms

II

Algae are classified by their pigments, food pigments, food reserves, morphology, and method of reporduction. Recent classification schemes recognize eight major groups (phyla).

A Blue-green (Cyanophyta)

B Green (Chlorophyta)

C Yellow-brown (Chrysophyta)

D Euglenoid (Euglenophyta)

E Dinoflagellates (Pyrrophyta)

F Red (Rhodophyta)

G Brown (Phaeophyta)

H Chloromonads (Chloromonadophyta)

The most numerous freshwater algae are the yellow browns, greens, and blue-greens.

III BLUE-GREEN ALGAE

This group contains many organisms that form nuisance blooms in eutrophic waters in later summer. They are considered to be the most primitive algae, and are similar to the bacteria in many respects. They lack many of the basic cell structures found in other algae and terrestrial plants, and have no sexual reproduction or motile forms. Many have the ability to fix nitrogen.

A The chlorophyll-bearing membranes are not organized to form distinct bodies (chloroplasts).

B The cells contain chlorophyll a, but are blue-green because of the presence of the blue pigment, phycocyanin (Table 1).

C The nucleus is not enclosed by a membrane.

D Reproduction is by cell division, colony fragmentation, and spore formation:

Spores of three types are observed.

1 Akinetes - enlarged, (often) thick-walled resting cells (see Anabaena).

2 Heterocysts - appear "empty", but have been observed to germinate (see Anabaena).

3 Endospores - formed by repeated division of protoplast in a cell (Chamaeosiphon).

TABLE 1

The distribution of pigments in the algal groups. ● = major pigment(s) of the group; ◑ = a pigment comprising less than half the total pigment content; ○ = present in small amounts. The data given by earlier authors varies considerably and the present table is compiled from tables in Fogg (1953), Strain (1958), Goodwin (1960), Egle (1960) and Haxo and O'Heocha (1960) with additions from their discussions.

Pigments \ Algal groups	Chlorophyceae	Siphonales[3]	Euglenophyceae	Xanthophyceae	Chrysophyceae	Bacillariophyceae	Phaeophyta	Dinophyceae	Rhodophyta	Cyanophyta	REMARKS
Chlorophylls											
Chlorophyll *a*	●	●	●	●	●	●	●	●	●	●	
Chlorophyll *b*	◑	◑	○								
Chlorophyll *c*						○	○	○			
Chlorophyll *d*									○*		*But not in all genera
Chlorophyll *e*				○							
Carotenes											
α-Carotene	±○	●				±○	±○		±○		
β-Carotene	●	○	●[4]	●	●	●	○	●	●	●	
γ-Carotene	○										Not in higher plants
ε-Carotene	?	○				○*					*In *Navicula torquatum*
Flavacene										○	
Xanthophylls											
Lutein	●	○	●[4]	○	○	?	±○		◑	±○	
Zeaxanthin	○	○?	?						±○	○	
Violoxanthin	○	○		○			○		○*		*Taraxanthin?
Flavoxanthin							±○				
Neoxanthin	○	○	○	○			○				
Siphonein		◑									
Siphonoxanthin		○									
Fucoxanthin				○	○	◑	●[2]				Occurs as a chromoprotein
Neofucoxanthin (A and B)						○	○				
Diatoxanthin						○	○				
Diadinoxanthin						○	○	○			
Neodiadinoxanthin								○			
Dinoxanthin								○			
Neodinoxanthin								○			
Peridinin								◑			
Neoperidinin								○			
Myxoxanthin (= Echinenone)										◑	Also syn Aphanin and Calorhodin
Myxoxanthophyll										◑	
Oscilloxanthin										○	
Astaxanthin	○*		○[3]								*In *Haematococcus*, *Brachiomonas* and *Protosiphon*
Unknown xanthophyll			○								
Phycobilins											
R-phycoerythrin									●		
R-phycocyanin									○		
C-phycoerythrin										○	Also in Cryptomonads
C-phycocyanin										●	Also in Cryptomonads, but absorption peaks not the same
β-phycoerythrin									○*		*In *Porphyra naiadum*
Allophycocyanin									○*		*In *Porphyra naiadum*

1. 3–8 times the content of β-carotene and lutein, but gametes of *Fucus* almost entirely β-carotene
2. Sometimes in greater amount than β carotene
3. In large amounts possibly in the cytoplasm
4. Dominant in some species. The carotenoid contents of *Euglena* spp. are higher than other algae.
= Bryopsidophyceae.

*From: Round, F.E. 1966. The Biology of the Algae. Edward Arnold (Publishers) LTD. London

E For convenience, the phylum is divided into two groups:

1 Coccoid - cells variously shaped, solitary or in masses, non-filamentous

2 Filamentous - filaments solitary or in masses, branched or unbranched, sheathed or unsheathed; specialized cells may be present

F Important Genera:

Anacystis (Microcystis), Gleotrichia, Anabaena, Aphanizomenon, Schizothrix, and Oscillatoria.

IV GREEN ALGAE

This group possesses intracellular structures (such as chloroplasts) and chlorophyll a and b in the ratio 2:1, similar to higher plants.

A The cells are grass green because of the predominance of the chlorophylls over the carotenes and xanthophylls.

B Sexual reproduction and motile cells are common.

C These algae are commonly divided into three groups:

1 Coccoid - non-motile, solitary or colonial. Actinastrum, Ankistrodesmus, Coelastrium, Golenkinia, Oocystis, Pediastrum, Scenedesmus.

2 Filamentous - attached or planktonic, branched or unbranched, Ulothrix, Mougeotia, Spirogyra, Stigeoclonium, Cladophora.

3 Flagellated - 2-4 flagella, solitary or colonial

D Important Genera: Chlamydomonas, Pandorina, Eudorina, Volvox, Carteria

V YELLOW-BROWN

These algae are yellowish green to golden brown because of a predominance of carotenes and xanthophylls. This group is usually the dominant form in the plankton and periphyton. The phylum contains coccoid, filamentous, and flagellate forms of varying importance.

A Coccoid - includes the diatoms (see below)

B Filamentous - important genera include Tribonema, Vancheria

C Flagellates - flagella single or paired, equal or unequal in length; cells solitary or colonial; some cells are armored. Important genera: Chromulina, Chrysococcus, Kephrion, Synura, Dinobryon.

D Diatoms

This group generally contributes 70-80% of the phytoplankton and periphyton. The cell walls contain silica and possess distinctive markings. The chloroplasts contain chlorophyll a and c in nearly equal amounts, but appear yellow brown because of the predominance of β-carotene and fucoxanthin. The diatoms are generally divided into two major groups:

1 Centric

The cells are circular in cross-section; drum-shaped, cylindrical, or spindle-shaped in side view. Important genera include: Cyclotella, Stephanodiscus, Melosira, Rhizosolenia.

2 Pennate

The cells are usually rectangular in cross-section and boat-shaped, with transverse rows of linear markings. Important genera include: Fragilaria, Synedra, Asterionella, Navicula, Nitzschia, Gomphonema.

VI EUGLENOPHYTA

A This group contains chlorophyll a and b and consists primarily of motile forms that have a single flagellum.

B The common genera are Euglena, Phacus, and Trachelomonas.

VII PYRROPHYTA

A These algae are greenish tan to golden brown, and contain chlorophyll a and c. The dominant forms (Dinoflagellates) are unicellular, motile, and are encircled by a transverse or spiral groove. One of the two flagella lies in the groove and encircles the cell; the other extends backward from the cell. Some forms lack a cell wall, while others have thick walls which appear to be composed of many plates.

B The common genera include Gymnodinium, Glenodinium, Gonyaulax, Peridinium, and Ceratium.

VIII RHODOPHYTA

A This phylum is largely marine, and contains chlorophyll a, β-carotene, phycoerythrin, and phycocyanin. The common freshwater species are filamentous, attached forms, and are not found in the plankton.

B Important freshwater genera include the filamentous forms, Batrachospernum, Audouinella and Lemanea.

IX PHAEOPHYTA

The brown algae are almost entirely marine, and have no important freshwater forms. They contain chlorophyll a and c and β-carotene, but are brown because of the predominance of fucoxanthin and other xanthophylls. This group is usually macroscopic, and contains the large "sea weeds" or kelp.

X CHLOROMONDOPHYTA

This is a small group of miscellaneous, little-understood, protozoa-like forms. There are no common genera in this phylum.

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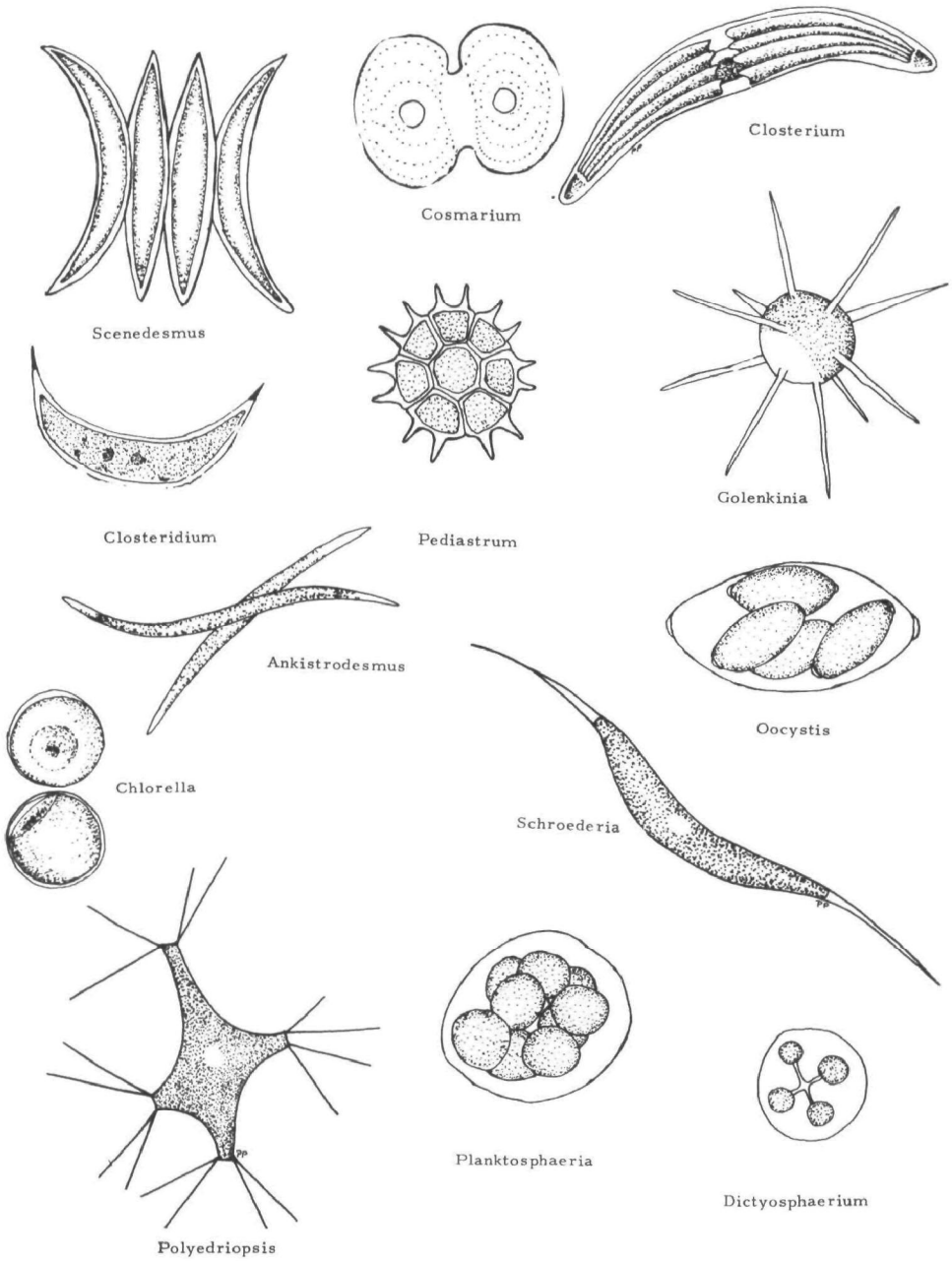
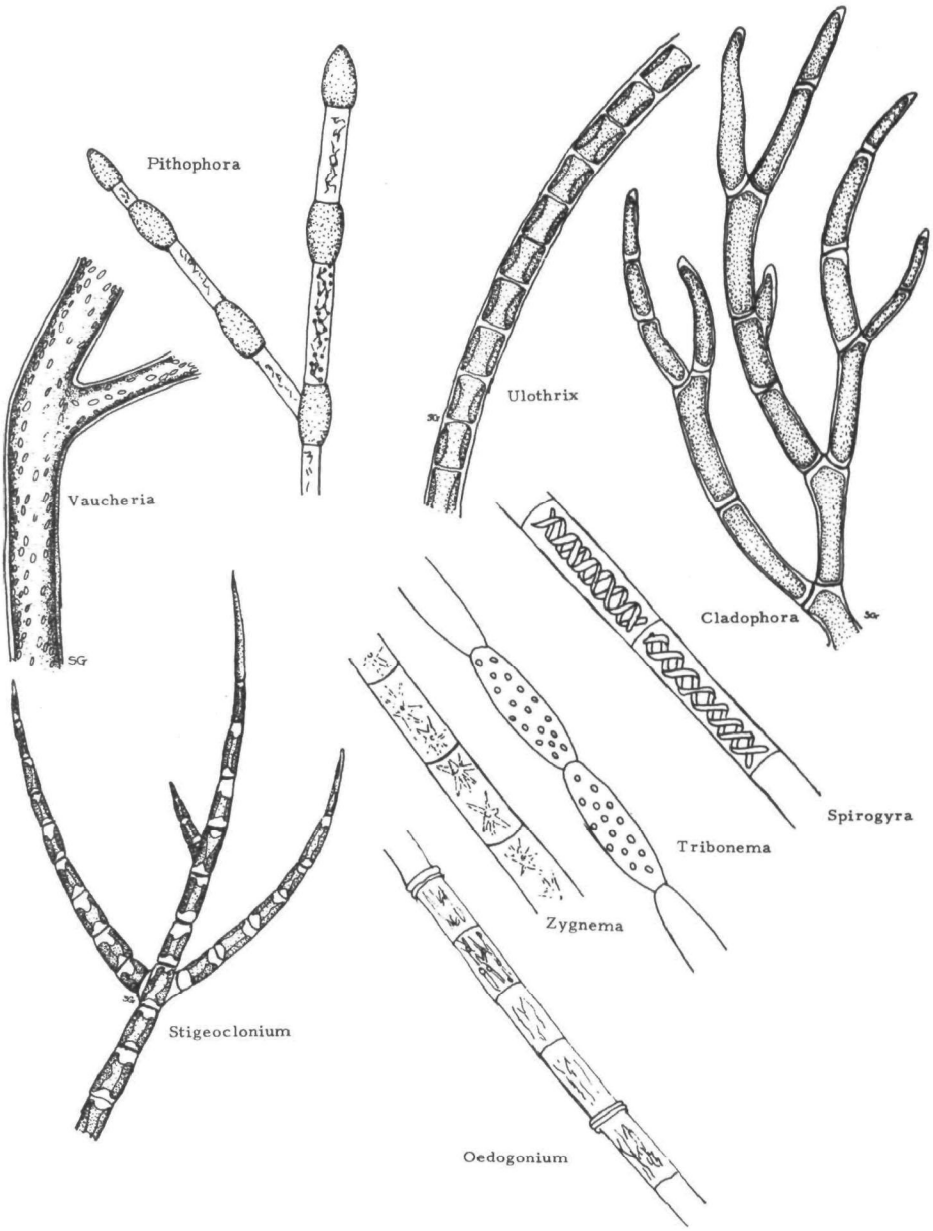
This outline was prepared by Dr. C.I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, FWPCA, 1014 Broadway, Cincinnati, OH 45202.

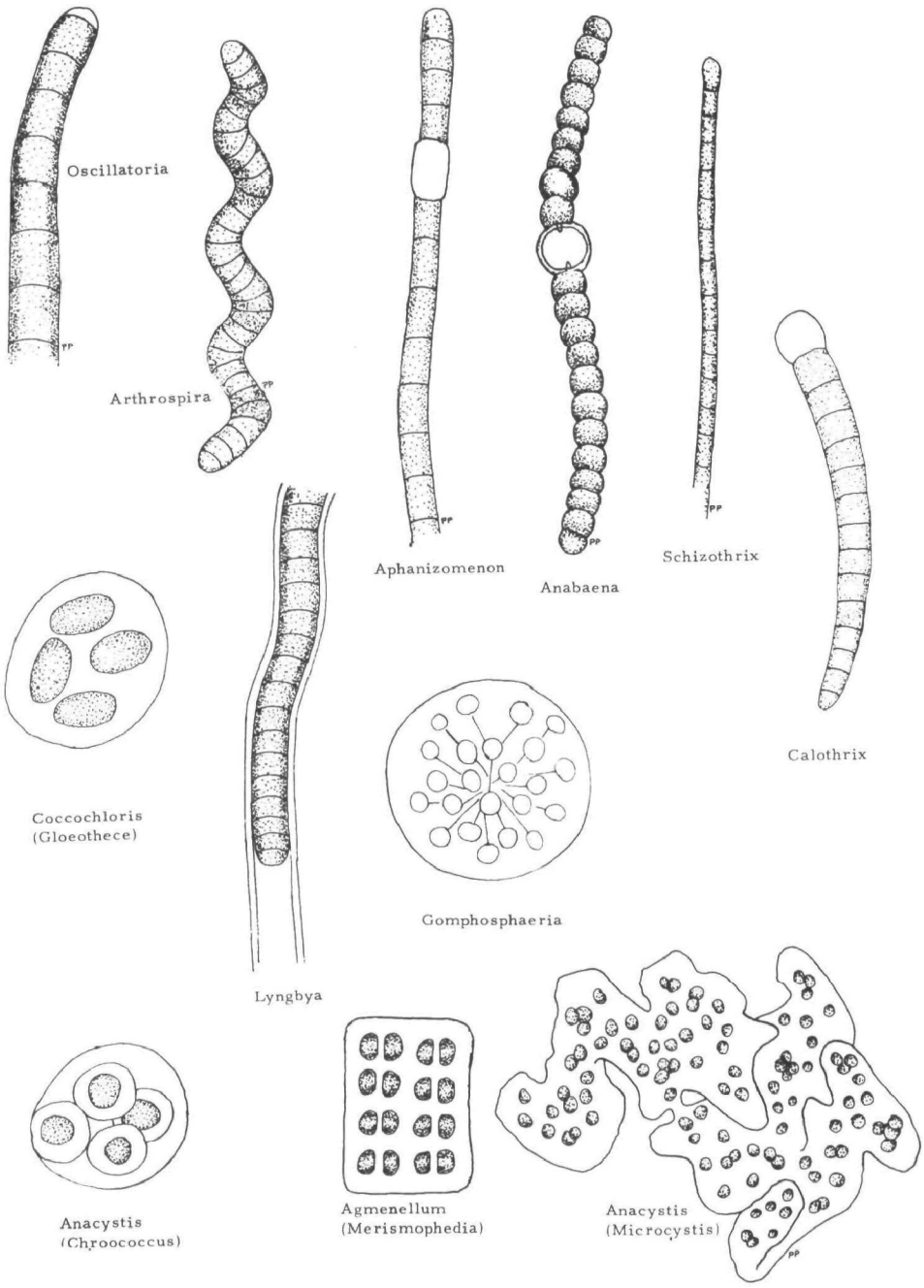
GREEN ALGAE, NON-FILAMENTOUS

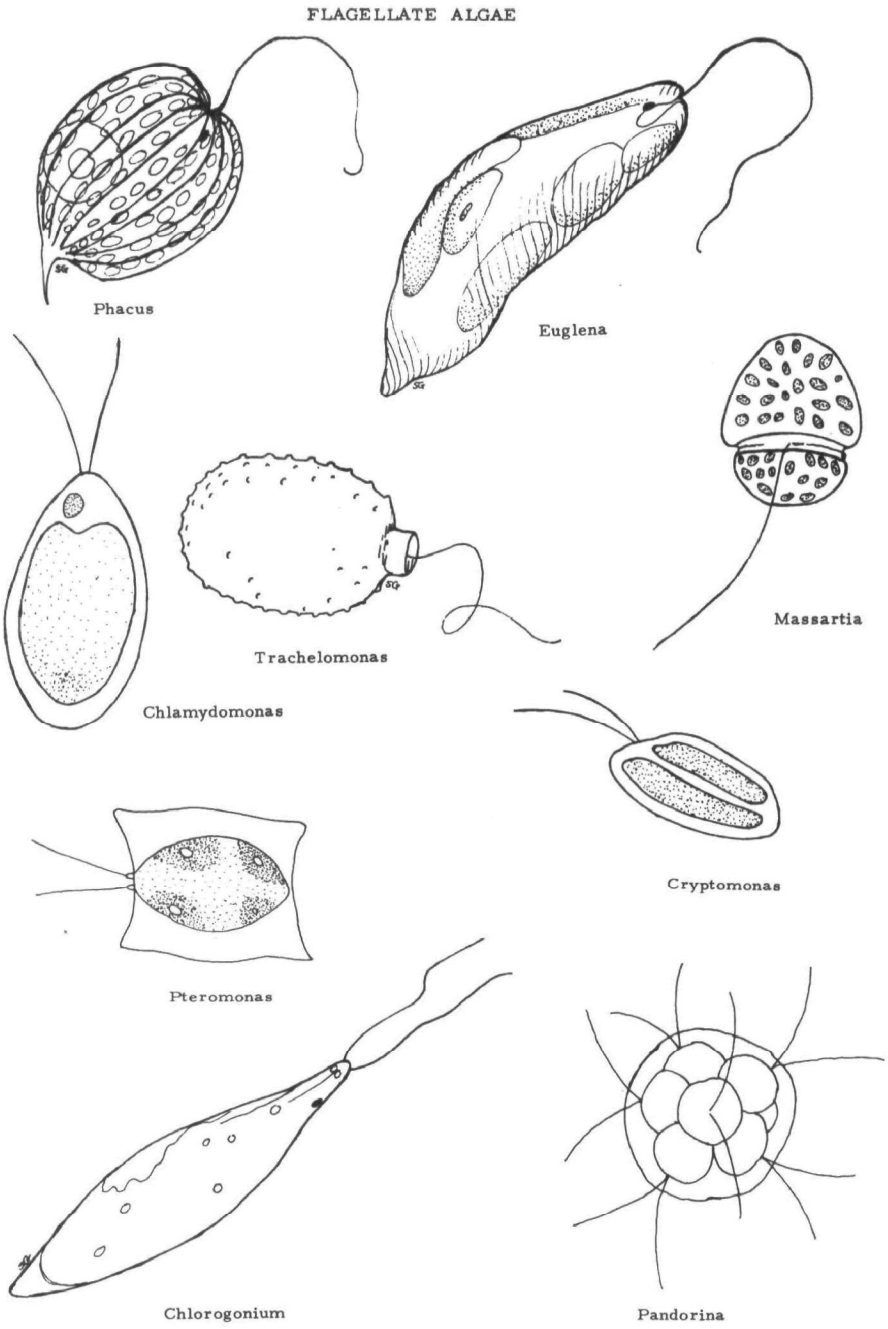


GREEN ALGAE, FILAMENTOUS

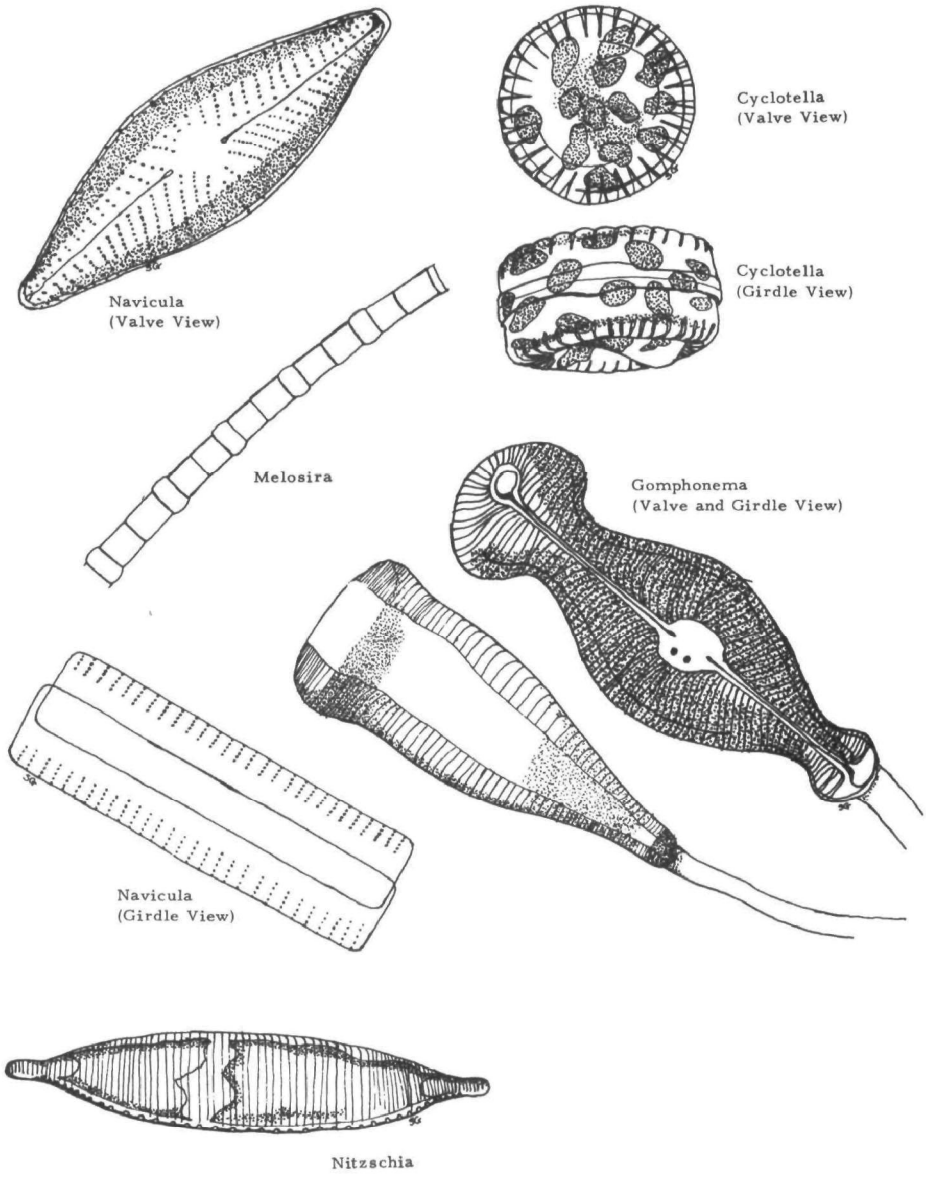


BLUE-GREEN ALGAE




FLAGELLATE ALGAE
Phacus
Euglena
Massartia
Trachelomonas
Chlamydomonas
Cryptomonas
Pteromonas
Chlorogonium
Pandorina

DIATOMS


Navicula
(Valve View)
Cyclotella
(Valve View)
Cyclotella
(Girdle View)
Melosira
Gomphonema
(Valve and Girdle View)
Navicula
(Girdle View)
Nitzschia

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 trichome, 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, nonmotile, 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 mucous 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.

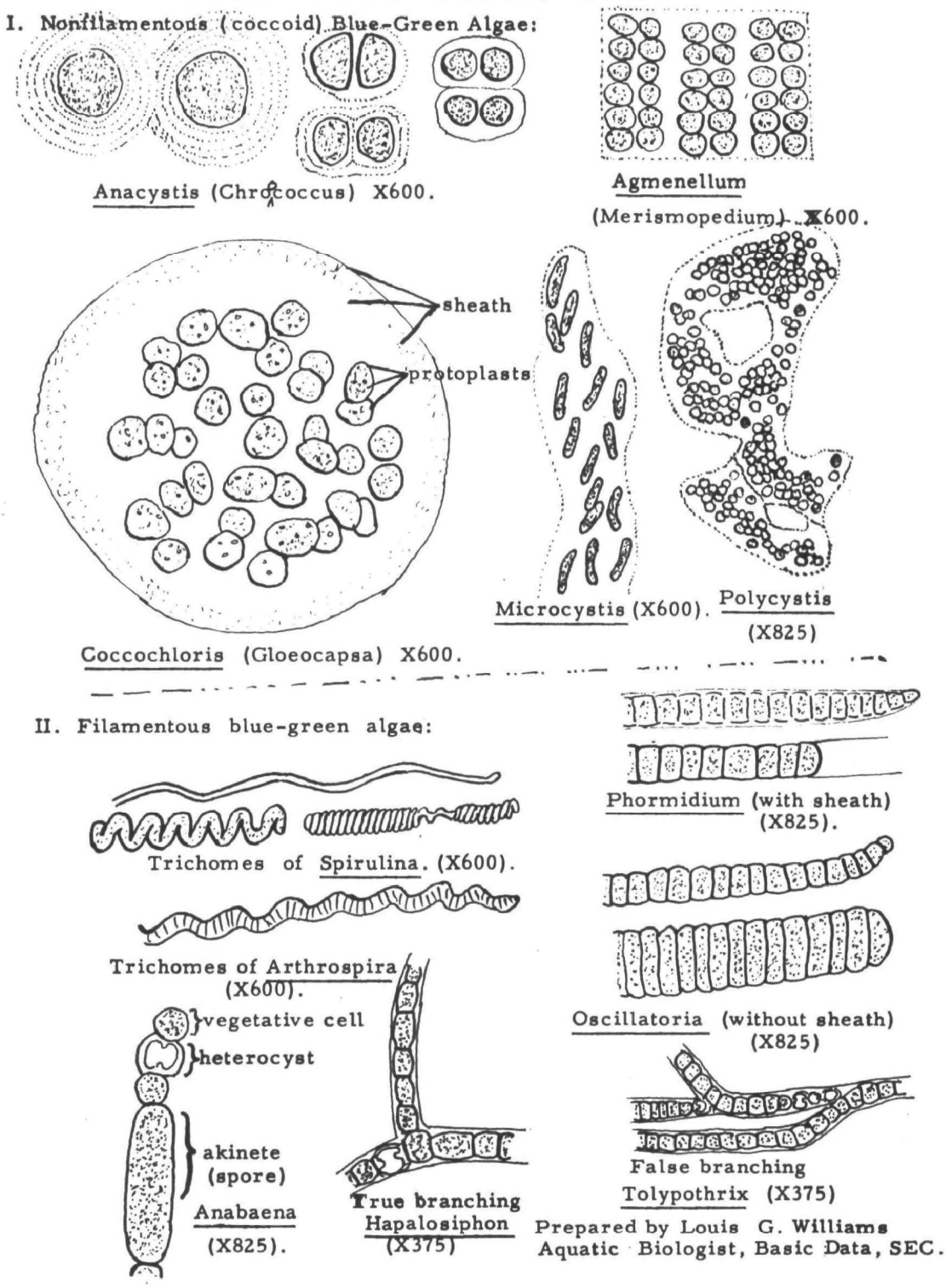
C Aphanizomenon is a strictly planktonic filamentous form.

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
I. Nonfilamentous (coccoid) Blue-Green Algae:
Anacystis (Chroococcus) X600.
Agmenellum
(Merismopedium) X600.
sheath
protoplasts
Coccochloris (Gloeocapsa) X600.
Microcystis (X600).
Polycystis
(X825)
II. Filamentous blue-green algae:
Trichomes of Spirulina. (X600).
Trichomes of Arthrospira
(X600).
vegetative cell
heterocyst
akinete
(spore)
Anabaena
(X825).
True branching
Hapalosiphon
(X375)
Phormidium (with sheath)
(X825).
Oscillatoria (without sheath)
(X825)
False branching
Tolypothrix (X375)
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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This outline was prepared by L. G. Williams, Formerly Aquatic Biologist, Aquatic Biology Activities, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

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. rufesceus 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.

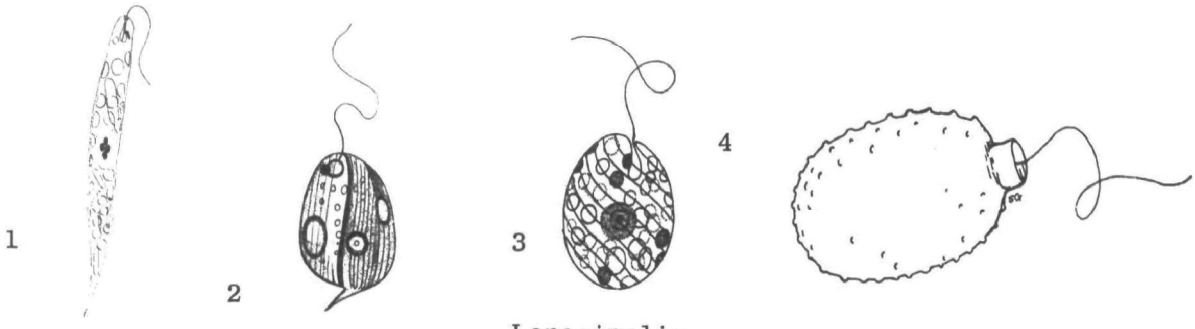
c Conspicuous eyespot usually present.

d Taxonomy of the group is involved.

e D. sertularia may clog filters.

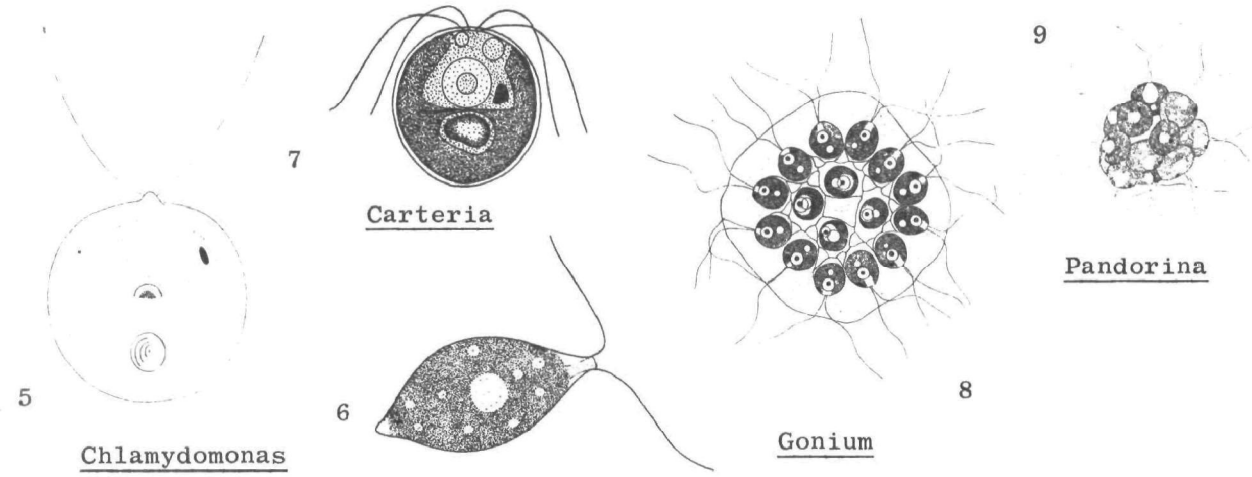
f D. divergens may cause a fishy odor.

(fig 1 - 13 from Lackey and Callaway)



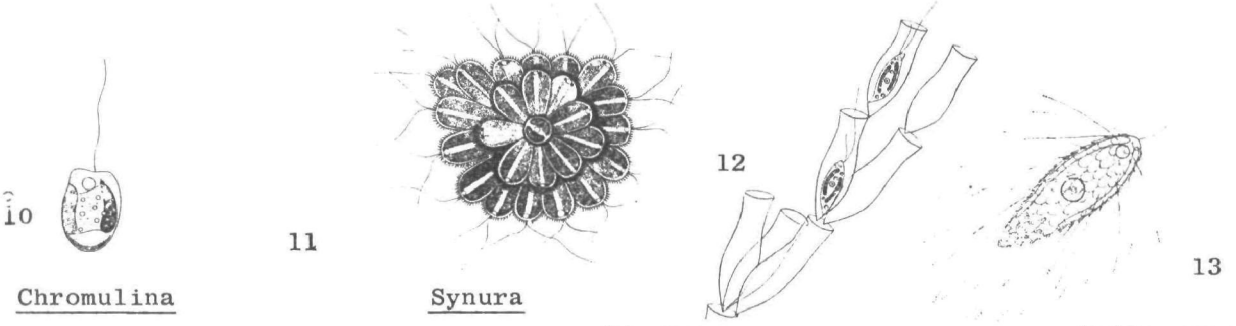
1 Euglena
2 Phacus
3 Lepocinclis
4 Trachelomonas

GREEN EUGLENOIDS



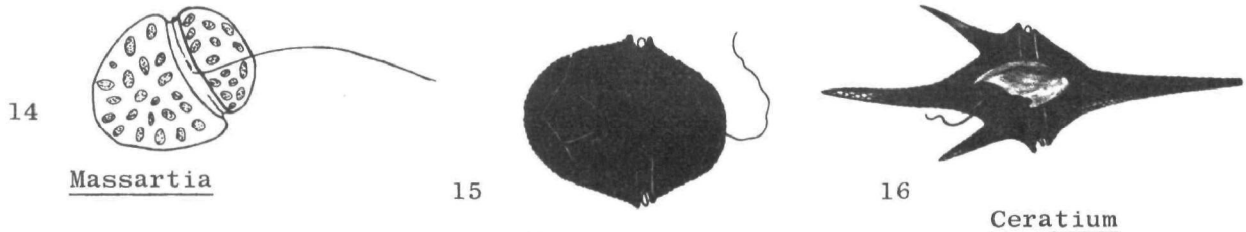
5 Chlamydomonas
6 Chlorogonium
7 Carteria
8 Gonium
9 Pandorina

GREEN PHYTOMONADS



10 Chromulina
11 Synura
12 Dinobryon
13 Mallomonas

YELLOW CHRYSOMONADS



14 Massartia
15 Peridinium
16 Ceratium

YELLOW-BROWN DINOFLAGELLATES

AMS 3-70

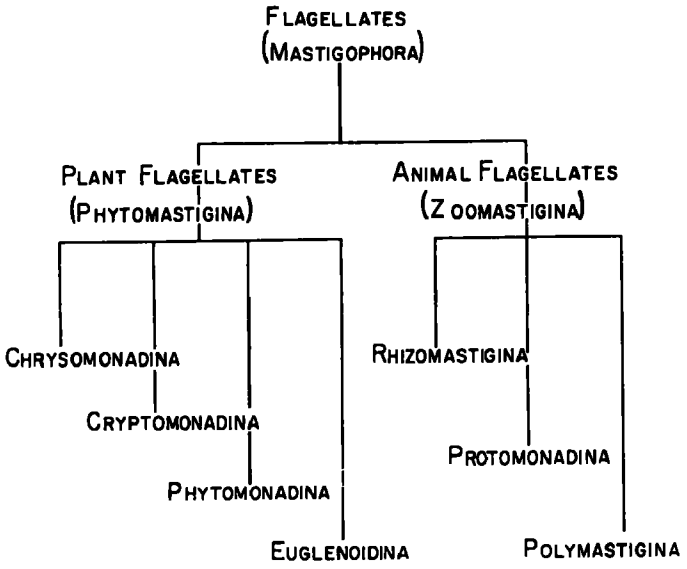


Figure 17 Phylogenetic Family Tree of the Flagellates
(from Calaway and Lackey)

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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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

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

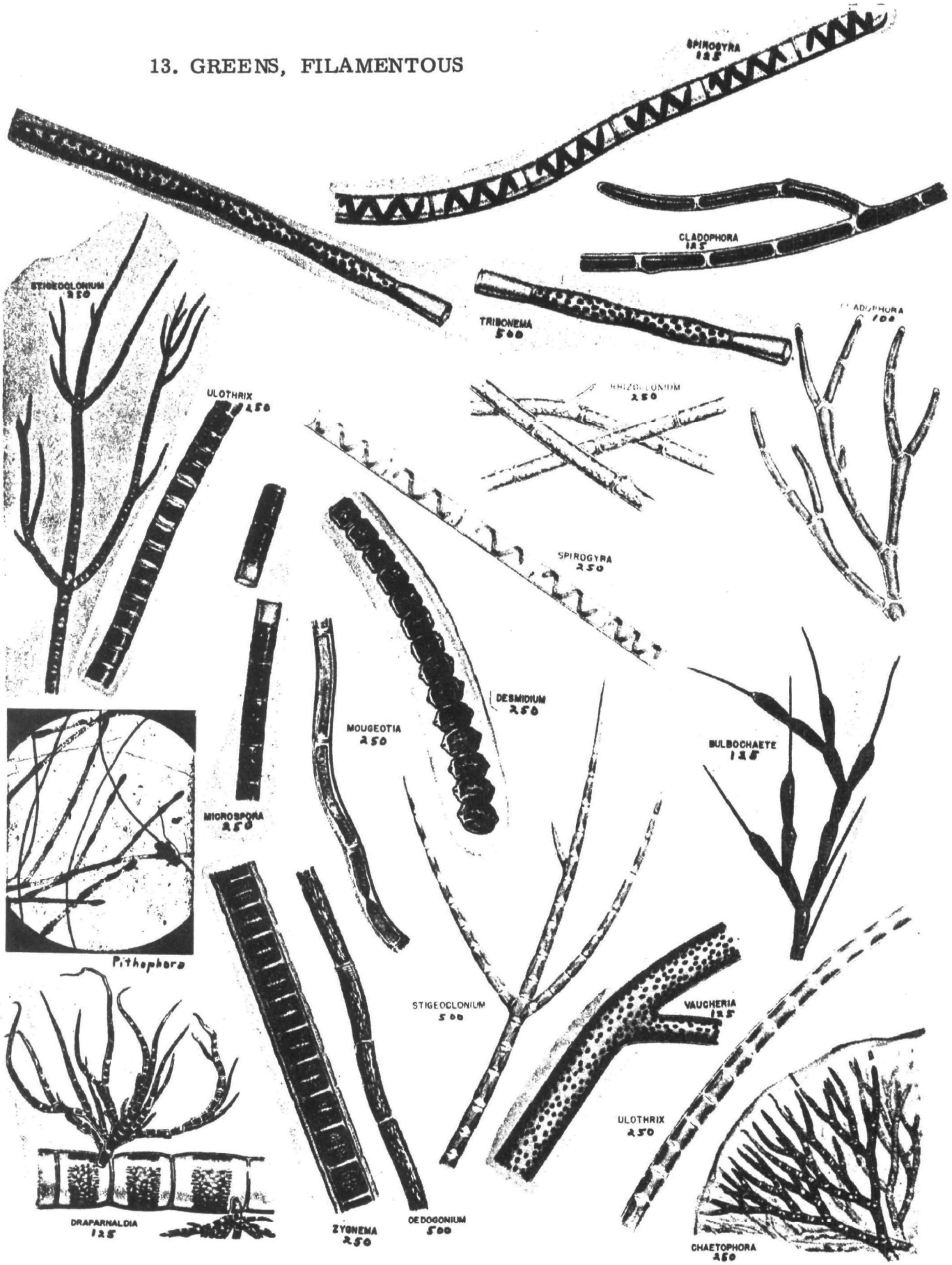
I Tribonemataceae

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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This outline was prepared by C.M. Palmer, Former Aquatic Biologist, In Charge, Interference Organisms Studies, Microbiology Activities, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

COCCOID GREEN ALGAE[1]

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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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

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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This outline was prepared by C.M. Palmer, Former Aquatic Biologist, In Charge, Interference Organisms Studies, Microbiology Activities, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

FILAMENTOUS BACTERIA

I INTRODUCTION

There are a number of types of filamentous bacteria that occur in the aquatic environment. They include the sheathed sulfur and iron bacteria such as Beggiatoa, Crenothrix and Sphaerotilus, the actinomycetes which are unicellular microorganisms that form chains of cells with special branchings, and Gallionella, a unicellular organism that secretes a long twisted ribbon-like stalk. These filamentous forms have at times created serious problems in rivers, reservoirs, wells, and water distribution systems.

II BEGGIATOA

Beggiatoa is a sheathed bacterium that grows as a long filamentous form. The flexible filaments may be as large as 25 microns wide and 100 microns long. Transverse separations within the sheath indicate that a row of cells is included in one sheath. The sheath may be clearly visible or so slight that only special staining will indicate that it is present.

The organism grows as a white slimy or felted cover on the surface of various objects undergoing decomposition or on the surface of stagnant areas of a stream receiving sewage. It has also been observed on the base of a trickling filter and in contact aerators.

It is most commonly found in sulfur springs or polluted waters where H_2S is present. Beggiatoa is distinguished by its ability to deposit sulfur within its cells; the sulfur deposits appear as large refractile globules. When H_2S is no longer present in the environment, the sulfur deposits disappear. Dr. Pringsheim of Germany has recently proved that the organism can grow as a true autotroph obtaining all its energy from the oxidation of H_2S and using this energy to fix CO_2 into all material. It can also use certain organic materials if they are present along with the H_2S.

Faust and Wolfe, and Scotten and Stokes have grown the organism in pure culture in this country. Beggiatoa exhibits a motility that is quite different from the typical flagellated motility of most bacteria; the filaments have a flexible gliding motion. The only major nuisance effect of Beggiatoa known has been overgrowth on trickling filters receiving waste waters rich in H_2S. The normal microflora of the filter was suppressed and the filter failed to give good treatment. Removal of the H_2S from the water by blowing air through the water before it reached the filters caused the slow decline of the Beggiatoa and a recovery of the normal microflora. Beggiatoa usually indicates polluted conditions with the presence of H_2S rather than being a direct nuisance.

III ACTINOMYCETES AND EARTHY ODORS IN WATER

Actinomycetes are unicellular microorganisms, 1 micron in diameter, filamentous, non-sheathed, branching monopodially, and reproduced by fission or by means of special conidia. Their filamentous habit and method of sporulation is reminiscent of fungi. However, their size, chemical composition, and other characteristics are more similar to bacteria. These organisms may be considered as a group intermediate between the fungi and the bacteria. They require organic matter for growth but can use a wide variety of substances and are widely distributed.

Actinomycetes have been implicated as the cause of earthy odors in some drinking waters (Romano and Safferman, Silvey and Roach) and in earthy smelling substance has

been isolated from several members of the group by Gerber and Lechevalier. Safferman and Morris have reported on a method for the "Isolation and Enumeration of Actinomycetes Related to Water Supplies." But the actinomycetes are primarily soil microorganisms and often grow in fields or on the banks of a river or lake used for the water supply. Although residual clorination will kill the organisms in the treatment plant or distribution system, the odors often are present before the water enters the plant. Use of permanganate oxidation and activated carbon filters have been most successful of the methods tried to remove the odors from the water. Control procedures to prevent the odorous material from being washed into the water supply by rains or to prevent possible development of the actinomycetes in water rich in decaying organic matter is still needed.

IV FILAMENTOUS IRON BACTERIA

The filamentous iron bacteria of the Sphaerotilus-Lepthothrix group, Crenothrix, and Gallionella have the ability to either oxidize manganous or ferrous ions to manganic or ferric salts or are able to accumulate precipitates of these compounds within the sheaths of the organisms. Extensive growths or accumulations of the empty, metallic encrusted sheaths devoid of cells, have created much trouble in wells or water distribution systems. Pumps and back surge valves have been clogged with masses of material, taste and odor problems have occurred, and rust colored masses of material have spoiled products in contact with water.

Crenothrix polyspora has only been examined under the microscope as we have never been able to grow it in the laboratory. The organism is easily recognized by its special morphology. Dr. Wolfe of the University of Illinois has published photomicrographs of the organism.

Organisms of the Sphaerotilus-Leptothrix group have been extensively studied by many investigators (Dondero et. al., Dondero, Stokes, Waitz and Lackey, Mulder and van Veen, and Amberg and Cormack.) Under different environmental conditions the morphological appearance of the organism varies. The usual form found in polluted streams or bulked activated sludge is Sphaerotilus natans. This is a sheathed bacterium consisting of long, unbranched filaments, whereby individual rod-shaped bacterial cells are enclosed in a linear order within the sheath. The individual cells are 3-8 microns long and 1.2-1.8 microns wide. Sphaerotilus grows in great masses; at times in streams or rivers that receive wastes from pulp mills, sugar refineries, distilleries, slaughterhouses, or milk processing plants. In these conditions, it appears as large masses or tufts attached to rocks, twigs, or other projections and the masses may vary in color from light grey to reddish brown. In some rivers large masses of Sphaerotilus break loose and clog water intake pipes or foul fishing nets. When the cells die, taste and odor problems may also occur in the water.

Amberg, Cormack, and Rivers and McKeown have reported on methods to try to limit the development of Sphaerotilus in rivers by intermittant discharge of wastes. Adequate control will probably only be achieved once the wastes are treated before discharge to such an extent that the growth of Sphaerotilus is no longer favored in the river. Sphaerotilus grows well at cool temperatures and slightly low DO levels in streams receiving these wastes and domestic sewage. Growth is slow where the only nitrogen present is inorganic nitrogen; peptones and proteins are utilized preferentially.

Gallionella is an iron bacterium which appears as a kidney-shaped cell with a twisted ribbon-like stalk emanating from the concavity of the cell. Gallionella obtains its energy by oxidizing ferrous iron to ferric iron and uses only CO_2 and inorganic salts to form all of the cell material; it is an autotroph. Large masses of Gallionella may cause problems in wells or accumulate in low-flow low-pressure water mains. Super chlorination (up to 100 ppm of sodium hypochlorite for 48 hours) followed by flushing will often remove the masses of growth and periodic treatment

will prevent the nuisance effects of the extensive masses of Gallionella.

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This outline was prepared by R.F. Lewis, Bacteriologist, Microbiological Activities, Research and Development, Cincinnati Water Research Laboratory, FWPCA.

FUNGI

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°-22°C) and both seem incapable of extensive growth at temperatures of about 30°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.

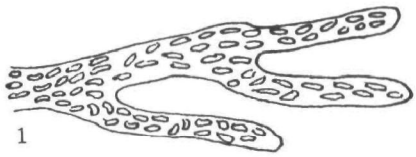
e Nutritive requirements

Sphaerotilus natans is able to utilize a wide variety of organic compounds, whereas L. lacteus does not assimilate simple

PLATE I

"SEWAGE FUNGUS" COMMUNITY

(Attached "filamentous" growths)



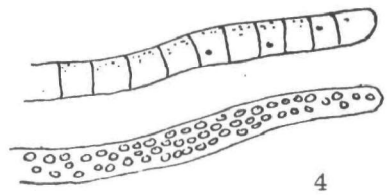
1

Zoogloea

3

Sphaerotilus natans

2

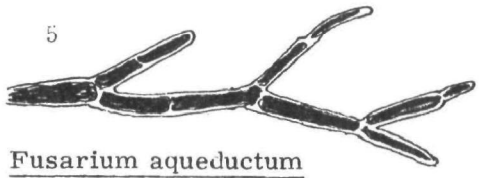


4

Beggiatoa alba

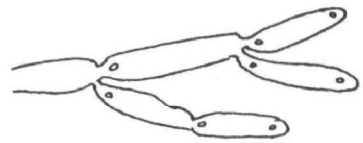
5 μ

BACTERIA



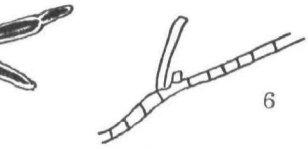
5

Fusarium aqueductum



7

Leptomitus lacteus

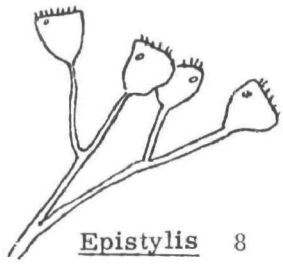


6

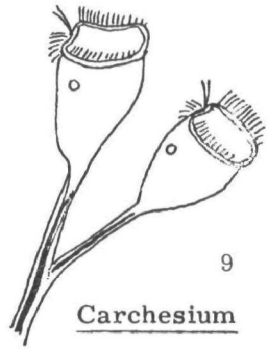
Geotrichum candidum

5 μ

FUNGI

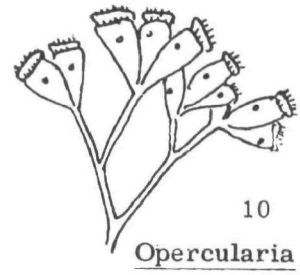


Epistylis 8



9

Carchesium



10

Opercularia

100 μ

PROTOZOA

PLATE II

REPRESENTATIVE FUNGI

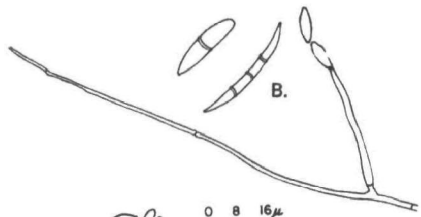
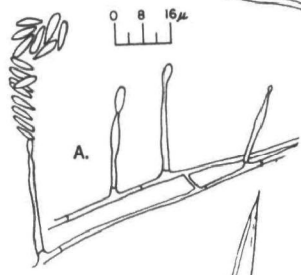


Figure 1

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.



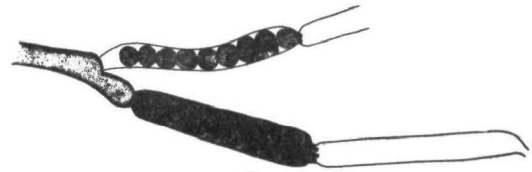


Figure 2

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.

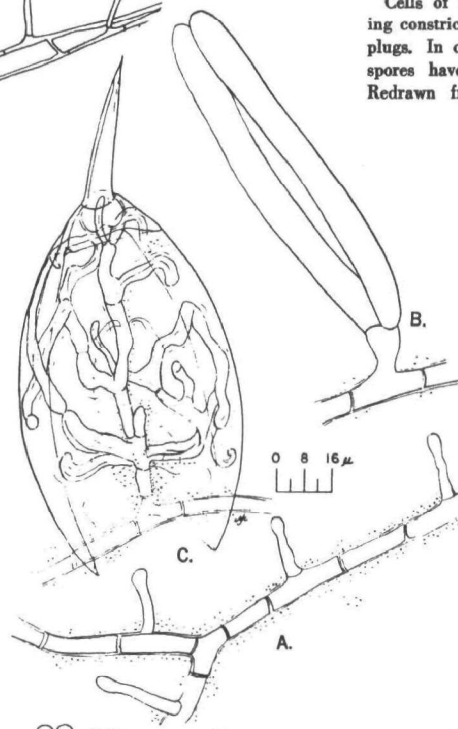


Figure 4

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.

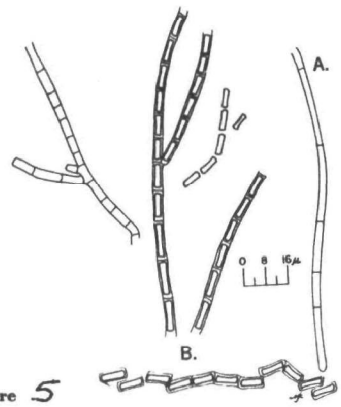
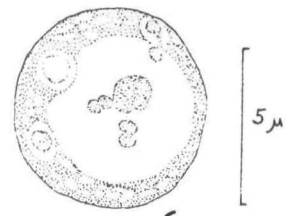


Figure 5

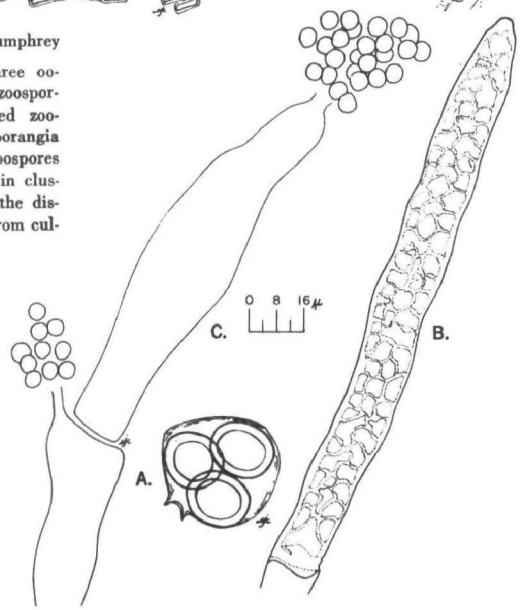
Achlya americana Humphrey

Oogonium 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



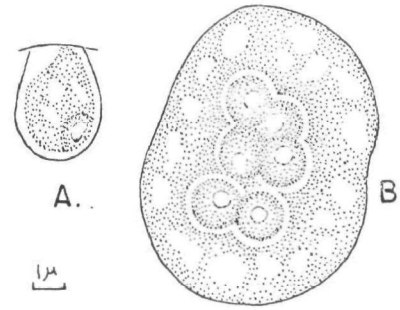


FIGURE 7 *Haplosporidium costale*. A—mature spore; B—early plasmodium.

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.

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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PROTOZOA, NEMATODES, AND ROTIFERS

I GENERAL CONSIDERATIONS

A Microbial quality constitutes only one aspect of water sanitation; microchemicals and radionuclides are attracting increasing amount of attention lately.

B Microbes considered here include bacteria, protozoa, and microscopic metazoa; algae and fungi excluded.

C Of the free-living forms, some are members of the flora and fauna of surface waters; others washed into the water from air and soil; still others of wastewater origin.

D Hard to separate "native" from "foreign" free-living microbes, due to close association of water with soil and other environments; generally speaking, bacteria adapted to water are those that can grow on very low concentrations of nutriment and zoomicrobes adapted to water are those that feed on algae.

E More species and lower densities of microbes in clean water and fewer species and higher densities in polluted water.

F Pollution-tolerance or nontolerance of microbes closely related to the DO level required in respiration.

G From pollution viewpoint, the following groups of microbes are of importance: Bacteria, Protozoa, Nematoda, and Rotifera.

II BACTERIA

A No ideal method for studying distribution and ecology of bacteria in freshwater.

B According to Collins,[9] Pseudomonas, Achrombacter, Alcaligenes, Chromobacterium, Flavobacterium, and Micrococcus are the most widely distributed and may be considered as indigenous to natural waters. Sulfur and iron bacteria are more common in the bottom mud.

C Actinomycetes, Bacillus spp., Aerogenes spp., and nitrogen-fixation bacteria are primarily soil dwellers and may be washed into the water by runoffs.

D E. coli, streptococci, and Cl. perfringens are true indicators of fecal pollution.

III PROTOZOA

A Classification

1 Single-cell animals in the most primitive phylum (Protozoa) in the animal kingdom.

2 A separate kingdom, Protista, to include protozoa, algae, fungi, and bacteria proposed in the 2nd edition of Ward-Whipple's Fresh-Water Biology.[10]

3 Four subphyla or classes:

a Mastigophora (flagellates) - Subclass Phytomastigina dealt with under algae; only subclass Zoomastigina included here; 4 orders:

1) Rhizomastigina - with flagellum or flagella and pseudopodia
2) Protomonadina - with 1 to 2 flagella; mostly free-living many parasitic
3) Polymastigina - with 3 to 8 flagella, mostly parasitic in elementary tract of animals and man
4) Hypermastigina - all inhabitants of elementary tract of insects

b Ciliophora or Infusoria (ciliates) - no pigmented members; 2 classes:

1) Ciliata - cilia present during the whole trophic life; containing majority of the ciliates

2) Suctoria - cilia present while young and tentacles during trophic life.

c Sarcodina (amoebae) - Pseudopodia (false feet) for locomotion and food-capturing; 2 subclasses:

1) Rhizopoda - Pseudopodia without axial filaments; 5 orders:

a) Proteomyxa - with radiating pseudopodia; without test or shell

b) Mycetozoa - forming plasmodium; resembling fungi in sporagium formation

c) Amoebina - true amoeba - forming lobopodia

d) Testacea - amoeba with single test or shell of chitinous material

e) Foraminifera - amoeba with 1 or more shells of calcareous nature; practically all marine forms

d Sporozoa - no organ of locomotion; amoeboid in asexual phase; all parasitic

B General Morphology

1 Zoomastigina:

Relatively small size (5 to 40 μ); with the exception of Rhizomastigina, the body has a definite shape (oval, leaf-like, pear-like, etc.); common members with 1 or 2 flagella and some with 3, 4, or more; few forming colonies; cytostome present in many for feeding.

2 Ciliophora:

Most highly developed protozoa; with few exceptions, a macro and a micronucleus; adoral zone of membranellae, mouth, and groove usually present in swimming and crawling forms, some with conspicuous ciliation of a disc-like anterior region and little or no body cilia (stalked and shelled forms); Suctoria nonmotile (attached) and without cytostome cysts formed in most.

3 Sarcodina:

Cytoplasmic membrane but no cell wall; endoplasm and ectoplasm distinct or indistinct; nucleus with small or large nucleolus; some with test or shell; moving by protruding pseudopodia; few capable of flagella transformation; freshwater actinopods usually sperical with many radiating axopodia; some Testacea containing symbiotic algae and mistaken for pigmented amoebae; cysts with single or double wall and 1 or 2 nuclei.

4 Sporozoa: to be mentioned later.

C General Physiology

1 Zoomastigina·

Free-living forms normally holozic; food supply mostly bacteria in growth film on surfaces or clumps relatively aerobic, therefore the first protozoa to disappear in anaerobic conditions and re-appearing at recovery; reproduction by simple fission or occasionally by budding.

2 Ciliophora:

Holozoic; true ciliates concentrating food particles by ciliary movement around the mouth part; suctoria sucking through tenacles; bacteria and small

algae and protozoa constitute main food under natural conditions; some shown in laboratory to thrive on dead organic matter and serum protein; not as aerobic as flagellates - some surviving under highly anaerobic conditions, such as Metopus; reproduction by simple fission, conjugation or encystation.

3 Sarcodina:

Holozoic; feeding through engulfing by pseudopodia; food essentially same as for ciliates; DO requirement somewhat similar to ciliates - the small amoebae and Testacea frequently present in large numbers in sewage effluent and polluted water; reproduction by simple fission and encystation.

IV NEMATODES

A Classification

1 All in the phylum Nemata (nonsegmented round worms); subdivided by some authors into two classes:

Secernentea - 3 orders:
(phasmids)

Tylenchida, Rhabditida, Strongylida, and Teratocephalida, with papillae on male tail, caudal glands absent.

Adenophora - 6 orders:
(aphasmids)

Araeolaimida, Dorylaimida, Chromdorida, Monhysterida, Enoplida, and Trichosyringida no papillae on male trail, caudal glands absent.

2 Orders encountered in water and sewage treatment - Free-living forms inhabitating sewage treatment plants are usually bacteria-feeders and those feeding on other nematodes; those inhabitating clean waters feeding on plant matters; they fall into the following orders:

3 Tylenchida - Stylet in mouth; mostly plant parasites; some feed on nematodes, such as Aphelenchoides.

4 Rhabditida - No stylet in mouth, caudal glands present, mostly bacteria-feeders, common genera: Rhabiditis, Diplogaster, Diplogasteroides, Monochoides, Pelodera, Panagrellus, and Turbatrix.

5 Dorylaimida - Relatively large nematodes; stylet in mouth, feeding on other nematodes and probably zoomicrobes; Dorylaimus common genus.

6 Chromadorida - Many marine forms, some freshwater dwellers feeding on algae; characterized by strong ornamentation of knobs, bristles or punctations in cuticle.

7 Monhysterida - Freshwater dwellers; esophago-intestinal valve spherical to elongated, ovaries single or paired, usually straight; common genus in water - Monhystera.

8 Enoplida - Head usually with a number of setae, Cobb reported one genus, Mononchulus, in sand filters in Washington, D.C.

B General Morphology

Round, slender, nonsegmented (transverse markings in cuticle of some) worms; some small (about $\frac{1}{2}$ mm long, as Tricephalobus), many 1 to 2 mm long (Rhabditis, Diplogaster, and Diplogasteriodes for instance), and some large (2 to 7 mm, such as Dorylaimus); sex separated but few parthenogenetic; complete elementary canal with elaborate mouth parts with or without stylet, complete reproductive system in each sex, no circulatory or respiratory system; complex nervous system with conspicuous nerve ring across oesophagus.

C General Physiology

1 Feeding - Most sewage treatment plant dwellers feeding on bacteria; others preying on protozoa, nematodes, rotifers,

etc., clean-water species apparently vegetarians; those with stylet in mouth use the latter to pierce the body of animal or plant and suck contents; metabolic waste mostly liquid containing ammonium carbonate or bicarbonate; enteric pathogens swallowed randomly with suspending fluid, hence remote possibility of sewage effluent-borne nematodes being pathogen-carriers.

2 Oxygen requirement - DO apparently diffused through cuticle into body; DO requirement somewhat similar to protozoa, Rhabditis tolerating reduced DO better than other Rhabditida members; all disappear under sepsis in liquid; some thrive in drying sludge.

3 Reproduction - Normal life cycle requires mating, egg with embryo formation, hatching of eggs inside or outside femals, 4 larval stages, and adult; few reproduce in the absence of males.

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 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 Digononta containing 1 order (Bdelloida) with 4 families, Philodinedae being the most important.

4 Class Monogononta comprising 3 orders: Notommatida (mouth not near center of corona) with 14 families, Floscularida Melicertida (corona with two wreaths of cilia and furrow between them) with 3 families; most import genera included in the order Notommatida: Brachionus, Keratella, Monostyla, Trichocerca, Asplanchna, Polyarthra, Synchaeta, Microcodon; common genera under the order Floscullariaceae: Floscularia, and Atrochus. Common genera under order Melicertida: Limnias and Conochilus.

5 Unfortunately orders and families of rotifers partly 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 ciliary movement on 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 non-tolerant 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 enertic pathogens.

VII EXAMINATION OF WATER FOR MICROBES

A Bacteria - not dealt here.

B Protozoa and rotifers - should be included in examination for planktonic microbes.

C Nematodes

D Laboratory Apparatus[3]

1 Sample Bottles - One-gallon glass or plastic bottles with metal or plastic screw caps, thoroughly washed and rinsed three times with distilled water.

2 Capillary Pipettes and Rubber Bulbs - Long (9 in.) Pasteur capillary pipettes and rubber bulbs of 2 ml capacity.

3 Filtration Unit - Any filter holder assembly used in bacteriological examination.[1] The funnel should be at least 650 ml and the filter flask at least 2 liter capacity.

4 Filter Membranes - Millipore SS (SS 047 MM) type membranes or equivalent.

5 Microscope - Binocular microscope with 10X eyepiece, 4X, 10X, and 43X objectives, and mechanical stage.

E Collection of Water Samples

Samples are collected in the same manner as those for bacteriological examination,[1] except that a dechlorinating agent is not needed. One-half to one gallon samples are collected from raw water and one-gallon samples from tap water. Refrigeration is not essential and samples may be transported without it unless examination is to be delayed for more than five days.

F Concentration of Samples

1 One gallon of tap water can usually be filtered through a single membrane within 15 minutes unless the water has turbidity. At least one gallon of sample should be used in a single examination. Immediately after the last of the water has disappeared through the membrane, the suction line is disconnected and the membrane placed on the wall of a clean 50 to 100 ml beaker and flushed repeatedly with about 2 ml of sterile

distilled water with the aid of a capillary pipette and a rubber bulb. The concentrate is then pipetted into a clean Sedgewick-Rafter Counting Cell and is ready for examination.

2 In concentration of raw water samples having visible turbidity, two to four membranes may be required per sample, with filtration through each membrane being limited to not more than 20 minutes. Samples ranging from 500 ml to 2 liters may be filtered, depending on whether turbidity is high or low. After filtration the membranes are placed on the walls of separate beakers and washed as above. To prevent the particulates from obscuring the nematodes, the washing from each filter is examined in a separate counting chamber.

G Direct Microscopic Examination

Each counting chamber containing the filter concentrate is first examined under a 4X objective. Unless the concentrate contains more than 100 worms, the whole cell area is surveyed for nematodes, with respect to number, developmental stage, and motility. When an object having an outline resembling that of a nematode is observed, it is re-examined under a 10X objective for anatomical structures, unless the object exhibits typical nematode movement, which is sufficient for identifying the object as a nematode. When the concentrate contains more than 100 worms, the worm density can be estimated by counting the number of worms in representative microscopic fields and multiplying the average number of worms per field by the number of fields in the cell area. The nematode density may be expressed as number of worms per gallon with or without differentiation as to adult or larval stages or as to viability.

H General Identification of Nematodes

1 While actively motile nematodes can be readily recognized by any person who has some general concept of microscopic animals, the nonmotile or sluggishly motile nematodes may be confused with root fibers, plant filaments of various types, elongated ciliates such as Homalozoon vermiculare, or segments of appendages of small crustacea. To facilitate a general identification of nematodes, the gross morphology of three of the free-living nematodes that are frequently found in water supplies is shown in the attached drawing. The drawing provides not only the general anatomy for recognition of nematodes but also most of the essential structures for guidance to those who want to use the "Key to Genera" in chapter No. 15 on Nemata by B. G. Chitwood and M. W. Allen in the book, Fresh Water Biology.[10]

2 Under normal conditions, practically all nematodes seen in samples of finished water are in various larval stages and will range from 100 to 500 microns in length and 10 to 40 microns in width. Except in the fourth (last) stage, the larvae have no sexual organs but show other structural characteristics.

3 If identification of genera is desired, the filter washings are centrifuged at 500 rpm for a few minutes. The supernate is discarded, except a few drops, and the sediment is resuspended in the remaining water. A drop of the final suspension is examined under both 10X and 43X objectives for anatomical characteristics without staining, and for supplementary study of structures the rest is fixed in 5% formalin or other fixation fluid and stained according to instructions given in Chitwood and Allen's Chapter on Nemata,[7] Goodey's Soil and Freshwater Nematodes[11] or other books on nematology.

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.

IX CONTROL

A Chlorination of effluent

B Prolongation of detention time of effluent

C Elimination of slow sand filters in nematode control.

LIST OF COMMON ZOOLOGICAL ORGANISMS FOUND IN SEWAGE TREATMENT PROCESS - TRICKLING FILTERS

PROTOZOA

Sarcodina - Amoebae

Amoeba proteus, A radiosa

Hartmannella Spp

Arcella Vulgaris

Noegleria gruberi

Actinophrys Sol

FLAGELLATA

Bodo caudatus

Pleuromonas jaculans

Oikomonas termo

Cercomonas longicauda

Peranema trichophorium

Swimming type

Ciliophora:

Colpidium colpoda

Colpoda cuculus

Glaucoma pyriformis

Paramecium candatum; 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)

Crawling type

Euplotes patella

Stylonychia mylitus

Urostyla Spp.

Oxytricha Spp.

NEMATODA

Diplogaster Spp.

Monochoides Spp.

Diplogasteroides Spp.

Rhabditis Spp.

Pelodera Spp.

Aphelenchoides Sp.

Dorylamus Sp.

Cylindrocorpus Sp.

Cephalobus Sp.

Rhabditolaimus Sp.

Monhystera Sp.

Trilobus Sp.

ROTATORIA

Diglena

Monstyla

Polyarthra

Philodina

Keratella

Brachionus

OLIGOCHAETA (bristle worms)

Aelosoma hemprichi

Aulophorus limosa

Tubifex tubifex

Lumbricillus lineatus

INSECT LARVAE

Chironomus

Psychoda Spp. (trickling filter fly)

ARTHROPODA

Lessertia Sp.

Porrhomma Sp.

Achoratus subuiaticus (collembola)

Folsomia Sp. (collembola)

Tomocerus Sp. (collembola)

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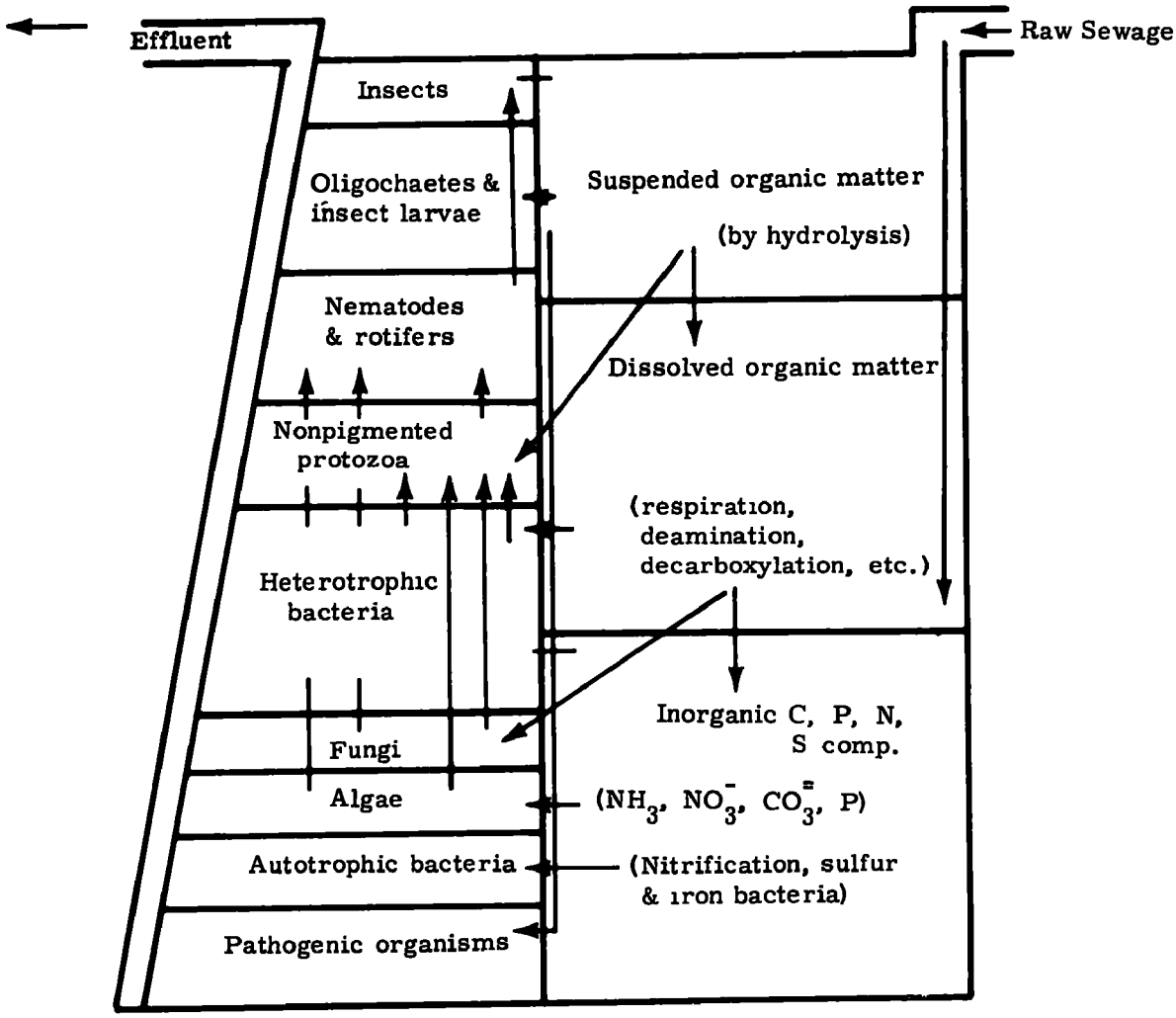
This outline was prepared by S. L Chang, Chief, Etiology, Disease Studies & Quality Control Laboratories, Water Supply & Sea Resources Program, National Center for Urban & Industrial Health.



Food Chain in Aerobic Sewage Treatment Processes

FREE-LIVING AMOEBAE AND NEMATODES

I FREE-LIVING AMOEBAE

A Importance of Recognizing Small, Free-Living Amoebae in Water Supplies

1 Commonly found in soil and natural, fresh waters - hence, frequently encountered in examination of raw water.

2 Cysts not infrequently found in municipal supplies - not pathogen carriers.

3 Cysts not to be confused with those of Endamoeba histolytica in water-borne epidemics.

4 Cysts useful in evaluation water treatment efficiency in removing or destroying cysts of pathogenic amoeba.

B Classification of Small, Free-Living Amoebae

1 Recognized classification based on characteristics in mitosis.

2 Common species fall into the following families and genera:

Family Schizopyrenidae: Genera Naegleria, Didascalus, and Schizopyrenus - first two being flagellate amoebae.

Family Hartmannellidae: Genera Hartmenella (Acanthamoeba)

3 How to prepare materials for studying mitosis - Feulgen stain

C Morphological Characteristics of Small, Free-Living Amoebae

1 Morphology of trophozoites - Ectoplasm and endoplasm with nucleus

2 Morphology of cysts - Single or double wall with or without pores

D Cultural Characteristics of Small, Free-Living Amoebae

1 How to cultivate these amoebae

2 Growth characteristics on plate cultures

3 Complex growth requirements for most of these amoebae

E Resistance of Amoebic Cysts to Physical and Chemical Agents

F Practical Use of Small, Free-Living Amoebae as Testing Organisms H. rhysodes, however, can grow in ordinary broth.

1 Culture-induced cysts for studying removal efficiency of flocculation and sand filtration and cysticidal efficiency of water disinfectants.

II FREE-LIVING NEMATODES

A Classification of Those Commonly Found in Water Supplies

1 Phasmidia: Genera Rhabditis, Diplogaster, Diplogasteroides, Cheilobus, Panagrolaimus

2 Aphasmidia: Genera Monhystera, Aphelenchus, Turbatrix (vinegar eel), Dorylaimus, and Rhabdolaimus

B Morphological Features

1 Phassids

2 Aphasmids

C Life Cycle

1 Methods of mating

2 Stages of development

3 Parthenogenesis

D Cultivation

1 Bacteria-fed cultures

2 Axenic cultures

E Occurrence in Water Supplies

1 Relationship between their appearance in finished water and that in raw water.

2 Frequency of occurrence in different types of raw water and sources .

3 Survival of human enteric pathogenic bacteria and viruses in nematodes.

4 Protection of human enteric pathogenic bacteria and viruses in nematode-carriers.

G Control

1 Chlorination of sewage effluent

2 Flocculation and sedimentation of water

3 Chlorination of water

4 Other methods of destruction

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This outline was prepared by Shih L. Chang, M.D., Chief, Etiology, Disease Studies & Quality Control Laboratories, Water Supply & Sea Resources Program, NCUIH, SEC.

ANIMAL PLANKTON

I INTRODUCTION

A Planktonic animals or zooplankton are found in nearly every major group of animals.

1 Truly planktonic species (euplankton) spend all or most of their active life cycle suspended in the water. Three groups are predominantly involved in fresh water; the protozoa, rotifers, and microcrustacea.

2 Transient planktonic phases such as floating eggs and cysts, and larval stages occur in many other groups.

B Many forms are strictly seasonal in occurrence.

C Certain rare forms occur in great numbers at unpredictable intervals.

D Techniques of collection, preservation, and identification strongly influence the species reported.

E In oceanographic work, the zooplankton is considered to include many relatively large animals such as siphonophores, ctenophores, hepteropods, pteropods, arrowworms, and euphausid shrimp.

F The plant-like or phytoplankton on the other hand are essentially similar in all waters, and are the nutritional foundation for the animal community.

II PHYLUM PROTOZOA

A The three typically free living classes, Mastigophora, Rhizopoda, and Ciliophora, all have planktonic representatives. As a group however, the majority of the phylum is benthic or bottom-loving. Nearly any of the benthic forms may occasionally be washed up into the overlying waters and thus be collected along with the euplankton.

B Class mastigophora, the nonpigmented zooflagellates.

These have frequently been confused with the phytomastigina or plant-like flagellates. The distinction is made here on the basis of the presence or absence of chlorophyll as suggested by Palmer and Ingram 1955.

(Note Figure: Nonpigmented, Non-Oxygen Producing Protozoan Flagellates in the outline Oxygen Relationships.)

1 Commonly encountered genera

Bodo

Peranema

2 Frequently associated with eutrophic conditions

C Class Rhizopoda - amoeboid protozoans

1 Forms commonly encountered as plankton:

Chaos	(Amoeba)
Arcella	Centropyxis
Difflugia	Heliozoa
Euglypha	

2 Cysts of some types may be encountered in water plants or distribution systems; rarely in plankton of open lakes or reservoirs.

D Class Ciliophora

1 Certain "attached" forms often found floating freely with plankton:

Vorticella

Carchesium

2 Naked, unattached ciliates. Halteria one of commonest in this group. Various heavily ciliated forms (holotrichs) may occur from time to time such as Colpidium, Enchelys, etc.

3 Ciliates protected by a shell or test (testaceous) are most often recorded from preserved samples. Particularly common in the experience of the National Water Quality Sampling Network are:

Codonella fluviatile

Codonella cratera

Tintinnidium (usually with organic matter)

Tintinnopsis

III PHYLUM ROTIFERA

A Some forms such as Anuraea cochlearis and Asplanchna pridonta tend to be present at all times of the year. Others such as Notholca striata, N. longispina and Polyarthra platyptera are reported to be essentially winter forms.

B Species in approximate order of descending frequency currently recorded by National Water Quality Sampling Network are:

Keratella cochlearis

Polyarthra vulgaris

Synchaeta pectinata

Brachionus quadridentata

Trichocerca longiseta

Rotaria sp.

Filinia longiseta

Kellicottia longispina

Pompholyx sp.

C Benthic species almost without number may be collected with the plankton from time to time.

IV PHYLUM ARTHROPODA

A Class Crustacea

1 The Class Crustacea includes the larger common freshwater euplankton. They are also the greatest planktonic consumers of basic nutrients in the form of phytoplankton, and are themselves the greatest planktonic contribution to the food of fishes. Most of them are herbivorous. Two groups, the cladocera and the copepods are most conspicuous.

2 Cladocera (Subclass Branchiopoda, Order Cladocera) or Water Fleas

a Life History

1) During most of the year, eggs which will develop without fertilization (parthenogenetic) are deposited by the female in a dorsal brood chamber. Here they hatch into minature adults which escape and swim away.

2) As unfavorable conditions develop, males appear, and thick-walled sexual eggs are enclosed in egg cases called ephippia which can often endure freezing and drying.

3) Sexual reproduction may occur at different seasons in different species.

4) Individuals of a great range of sizes, and even ephippia, are thus encountered in the plankton, but there is no "larval" form.

b Seasonal variation - Considerable variation may occur between winter and summer forms of the same species in some cases. Similar variation also occurs between arctic and tropical situations.

c Forms commonly encountered as open water plankton include:

Bosmina longirostris and others

Daphnia galeata and others

Other less common genera are:

Diaphanosoma, Chydorus, Sida, Acroperus, Ceriodaphnia, Bythotrephes, and the carnivorous Leptodora and Polyphemus.

d Heavy blooms of Cladocerans may build up in eutrophic waters.

3 The copepods (order Copepoda) are the perennial microcrustacea of open waters, both fresh and marine. They are the most ubiquitous of animal plankton.

a Cyclops is the genus most often found by the National Water Quality Sampling Network activities. Eucyclops, Paracyclops, Diaptomus, Canthocamptus, Epischura, and Limnocalanus are other forms reported to be planktonic.

b Copepods hatch into a minute characteristic larvae called a nauplius which differs considerably from the adults. After five or six moults, the copepodid stage is reached, and after six more moults, the adult. These larval stages are often encountered and are difficult to identify.

B Class Insecta

1 Only a single species of insect can be ranked as a true plankton, this is the midge fly Chaborus (approx. 8 spp, formerly Corethra).

2 The larva of this insect has hydrostatic organs that enable it to remain permanently suspended in the water.

3 It is usually found in the depths during the daytime, but comes to the surface at night.

V OCCASIONAL PLANKTERS

A While the protozoa, rotifers, and microcrustacea make up the bulk of the plankton, there are many other groups as mentioned above that may also occur. Locally or periodically these may be of major importance. Examples are given below.

B Phylum Coelenterata

1 Polyps of the genus Hydra may become detached and float about hanging from the surface film or floating detritus.

2 The freshwater medusa Craspedacusta occasionally appears in lakes or reservoirs in great numbers.

C Phylum Platyhelminthes

1 Minute Turbellaria (relatives of the well known Planaria) are sometimes taken with the plankton in eutrophic conditions. They are readily confused with ciliate protozoa.

2 Cercaria larvae of Trematodes (flukes) parasitic on certain wild animals, frequently appear in great numbers. When trapped in the droplets of water on a swimmer's skin, they attempt to bore in. Man not being their natural host, they fail. The resultant irritation is called "swimmer's itch". Some can be identified, but many unidentifiable species may be found.

3 In many areas of the world, cercaria larvae of human parasites such as the blood fluke Schistosoma japonicum may live as plankton, and penetrate the human skin directly on contact.

D Phylum Nemathelminthes

1 Nematodes (or nemas) or roundworms approach the bacteria and the blue-green algae in ubiquity. They are found in the soil and in the water, and in the air as dust. In both marine and fresh waters and from the Arctic to the tropics.

2 Although the majority are free living, some occur as parasites of plants, animals, and man, and some of these parasites are among out most serious.

3 With this distribution, it is obvious that they will occasionally be encountered as plankton. A more complete discussion of nematodes and their public health implications in water supplies will be found elsewhere (Chang, S L.).

E Additional crustacean groups sporadically met with in the plankton include the following:

1 Order Anostraca or fairy shrimps (formerly included with the two following orders in the Euphyllopoda) primarily planktonic in nature.

a Extremely local and sporadic, but when present, may be dominating.

b Artemia, the brine shrimp, can tolerate very high salinities.

c Very widely distributed, poorly understood.

2 Order Notostraca, the tadpole shrimps. Essentially southern and western in distribution.

3 Order Conchostraca, the clam shrimps. Widely distributed, sporadic in occurrence. Many local species.

4 Subclass Ostracoda, the seed shrimps. Up to 3 in. in length. Essentially benthic but certain species of Cypris, and Notodromas may occur in considerable numbers as plankton at certain times of the year.

5 Certain members of the large subclass Malacostraca are limnetic, and thus, planktonic to some extent.

a The scuds, (order Amphipoda) are essentially benthic but are sometimes collected in plankton samples around

weed beds or near shore. Nektoplanktonic forms include Pontoporeia and some species of Gammarus.

b The mysid, or opossum shrimps are represented among the plankton by Mysis relicta, which occurs in the deeper waters, large lakes as far north as the Arctic Ocean.

F The Class Archnoidea, Order Hydracarina (or Acari) the mites. Frequent in plankton tows near shore although Unionicola crassipes has been reported to be virtually planktonic.

G The phylum Mollusca is but scantily represented in the freshwater plankton, in contrast to the marine situation. Glochidia (ciliated) larvae are occasionally collected, and snails now and then glide out on a quiet surface film and are taken in a plankton net. An exotic bivalve Corbicula has a planktotrophic veliger stage.

H Eggs and other reproductive structures of many forms including fish, insects, and rotifers may be found in plankton samples. Special reproductive structures such as the statoblasts of bryozoa and sponges, and the ephippia of cladocerans may also be included.

I Adventitious and Accidental Plankters

Many shallow water benthic organisms may become accidentally and temporarily incorporated into the plankton. Many of those in the preceding section might be listed here, in addition to such forms as certain free living nematodes, small oligochaetes, and tardigrades, Collembola and other surface film livers are also taken at times but should not be mistaken for plankton. Fragments and molt skins from a variety of arthropods are usually observed.

Pollen from terrestrial or aquatic plants is often unrecognized, or confused with one of the above. Leaf hairs from terrestrial plants are also confusing to the uninitiated, they are sometimes mistaken for fungi or other organisms (and vice versa).

In flowing waters, normally benthic (bottom living) organisms are often found drifting freely in the stream. This phenomenon may be constant or periodic. When included in plankton collections, they must be reported, but recognized for what they are.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

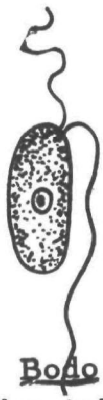
Phylum PROTOZOA

3/4

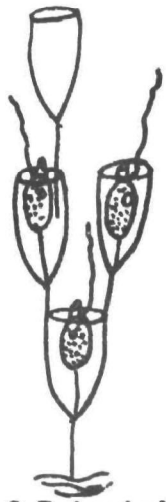
Free Living Representatives

I. Flagellated Protozoa, Class Mastigophora

Anthophysis
Pollution tollerant
6 μ

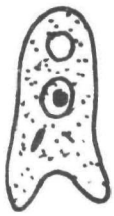


Bodo
Pollution tollerant
19 μ

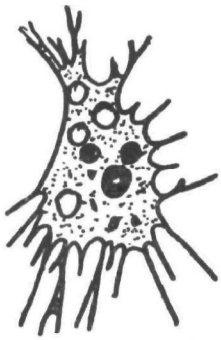


Colony of Poteriodendron
Pollution tollerant, 35 μ

II. Ameboid Protozoa, Class Sarcodina



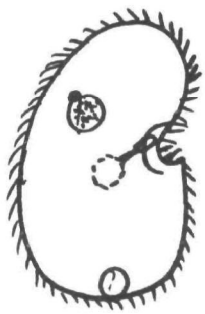
Dimastigamoeba
Pollution tollerant
10-50 μ



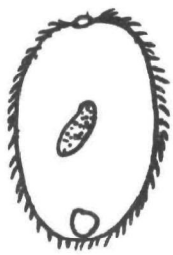
Nuclearia, reported to be intollerant of pollution, 45 μ.

Difflugia
Pollution tollerant
60-500 μ

III. Ciliated Protozoa, Class Ciliophora



Colpoda
Pollution tollerant
20-120 μ



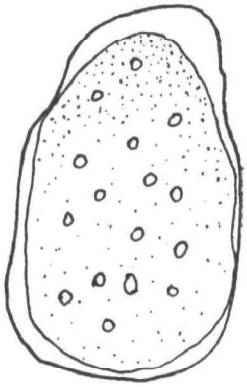
Holophrya, reported to be intollerant of pollution, 35 μ

Epistylis, pollution tollerant. Colonies often macroscopic.

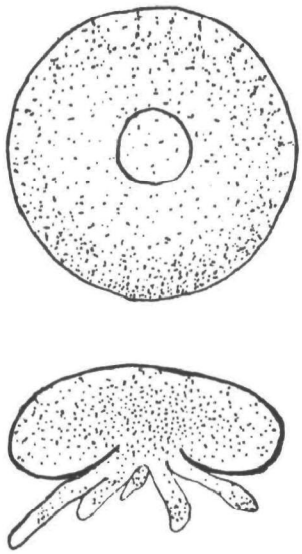
H.W. Jackson

PLANKTONIC PROTOZOA

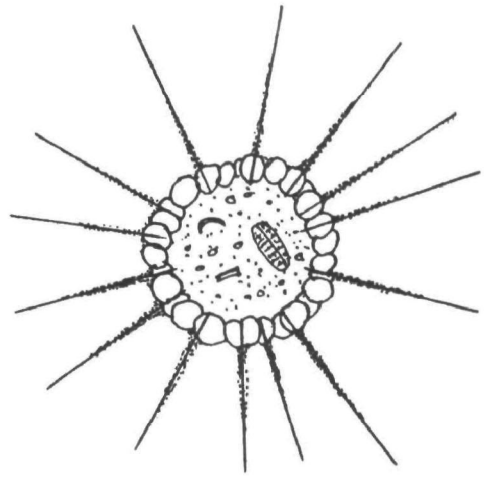
Peranema trichophorum



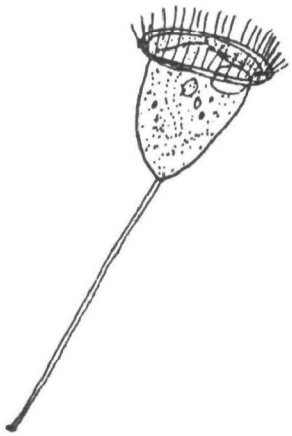
Chaos



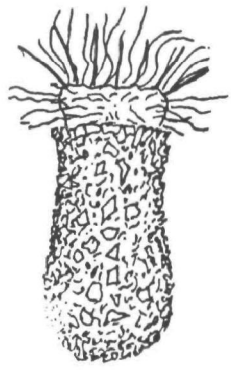
Arcella vulgaris



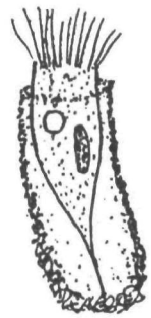
Actinosphaerium



Vorticella

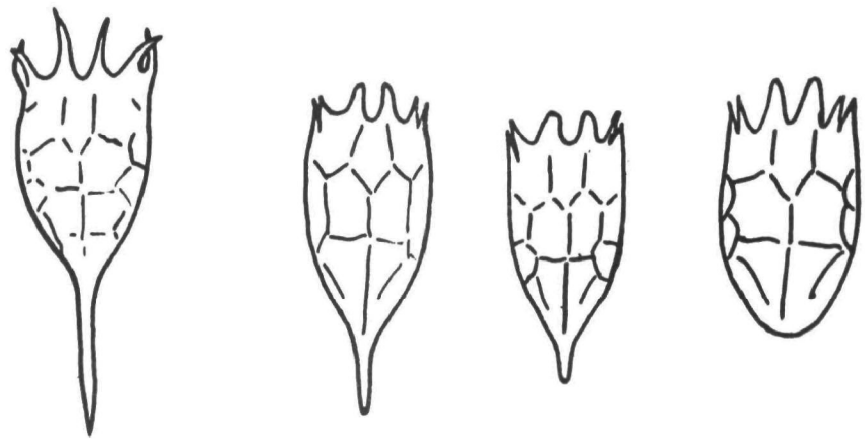


Codonella cratera

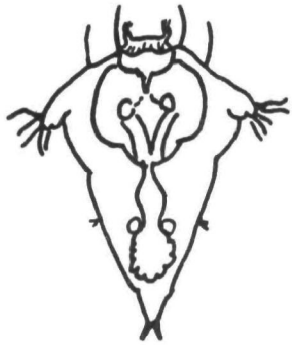


Tintinnidium fluviatile

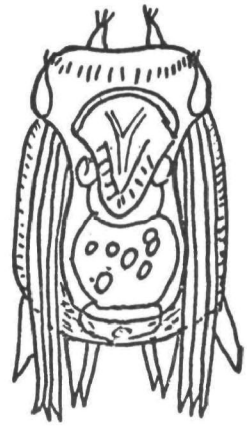
PLANKTONIC ROTIFERS



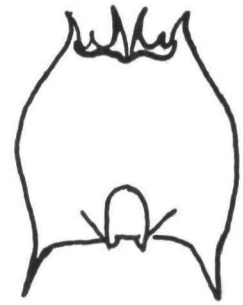
Various Forms of Keratella cochlearis



Synchaeta pectinata



Polygarthra vulgaris

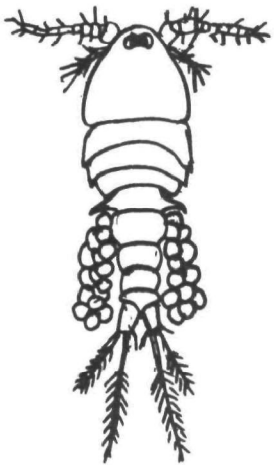


Brachionus quadridentata

Rotaria sp

SOME PLANKTONIC CRUSTACEANS

CRUSTACEANS

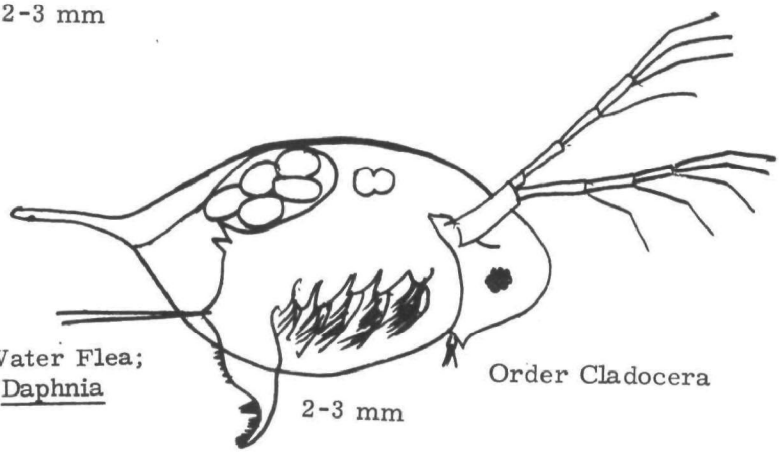


Copepod; Cyclops, Order Copepoda

2-3 mm

A Nauplius larva of a Copepod

1-5 mm

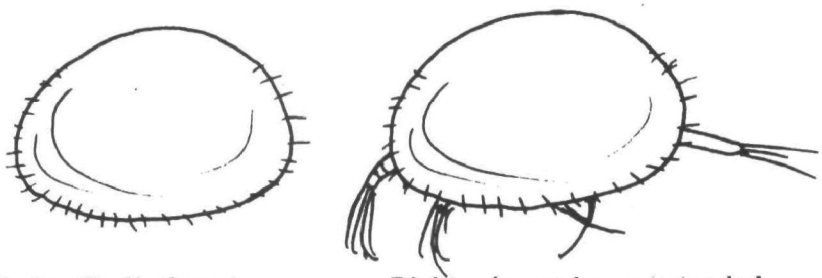


Water Flea;
Daphnia

2-3 mm

Order Cladocera

OSTRACODE



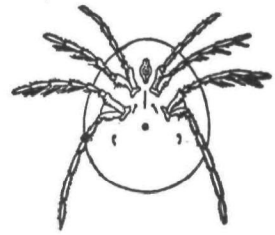
Left: Shell closed

Right: Appendages extended

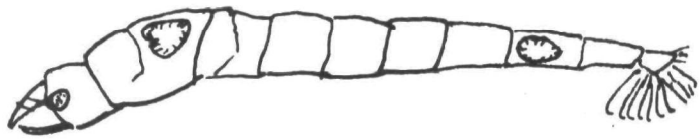
1-2 mm

PLANKTONIC ARTHROPODA

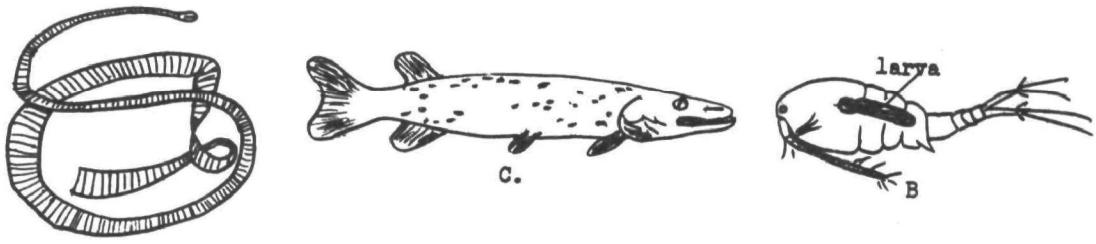
A mysid shrimp - crustacean



A water mite - arachnid



Chaoborus midge larva - Insect



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).

H. W. Jackson

LABORATORY EXERCISES

General Laboratory Instructions	15
Types of Algae	16
Identification of Diatoms	17
Identification of Animal Plankton	18

GENERAL LABORATORY INSTRUCTIONS

I GROUND RULES

A Students will be assigned to specific benches or seats.

B Certain items of equipment such as microscopes or other instruments will be assigned for the use of specific students or groups.

C Certain reference books and other items will be assigned to a "Central Stores" table from which they may be borrowed, and to which they should be returned when not in use. The person at whose desk they are located at the end of the period is responsible for returning them to Central Stores.

II FLASH CARDS

A A major objective of certain laboratory sessions is to become familiar with a given group or groups of organisms. This means associating names with organisms and/or parts. One of the most effective devices to accomplish this objective has been found to be the individually prepared "flash card."

This consists of a card (3X5, for example) with a sketch of an organism on one side, and its classification written on the other side. For later study, a pack of such cards is arranged with all sketches up, for example. Leaf through the pack. Your sketch will remind you of the organism you observed in laboratory or elsewhere. Think its name. If you are in doubt, turn the card over to check yourself. When you have learned to recognize all the sketches in the pack in hand, turn it over and go over the names, recalling in your mind the organisms (sketch) each refers to.

B A few sample flash cards will be furnished. You are herby instructed to prepare additional cards for yourself of as many different organisms as possible in every laboratory or field session where it is appropriate. The instructors will be glad to check your identification or other items on request but cannot take it upon themselves to call in or check all cards. Make frequent references to the "Finder." A supply of blank cards will be maintained at Central Stores.

C Identification represents an expenditure of time and effort. If this achievement is retained on a 3X5 card, then repetitious time in identification may be saved later. Partial identification or even no identification can be completed later, as one's competence and resource aids are enlarged. Code letters or numbers can be used on these unknowns and when identification becomes more complete, data may be more meaningful. Date collected, location, and selected known environmental parameters can enhance the usefulness of such a set, if these are added to each card (see Figure 1).

III LABORATORY SESSIONS

The laboratory sessions will not all be conducted in exactly the same way, as several different instructors will be involved. Where no special procedure is indicated at the end of the lecture outline covering a given group, the instructor will give verbal instructions on the spot.

IV PRINCIPAL OBJECTIVES OF THE FIRST LABORATORY SESSION WILL BE:

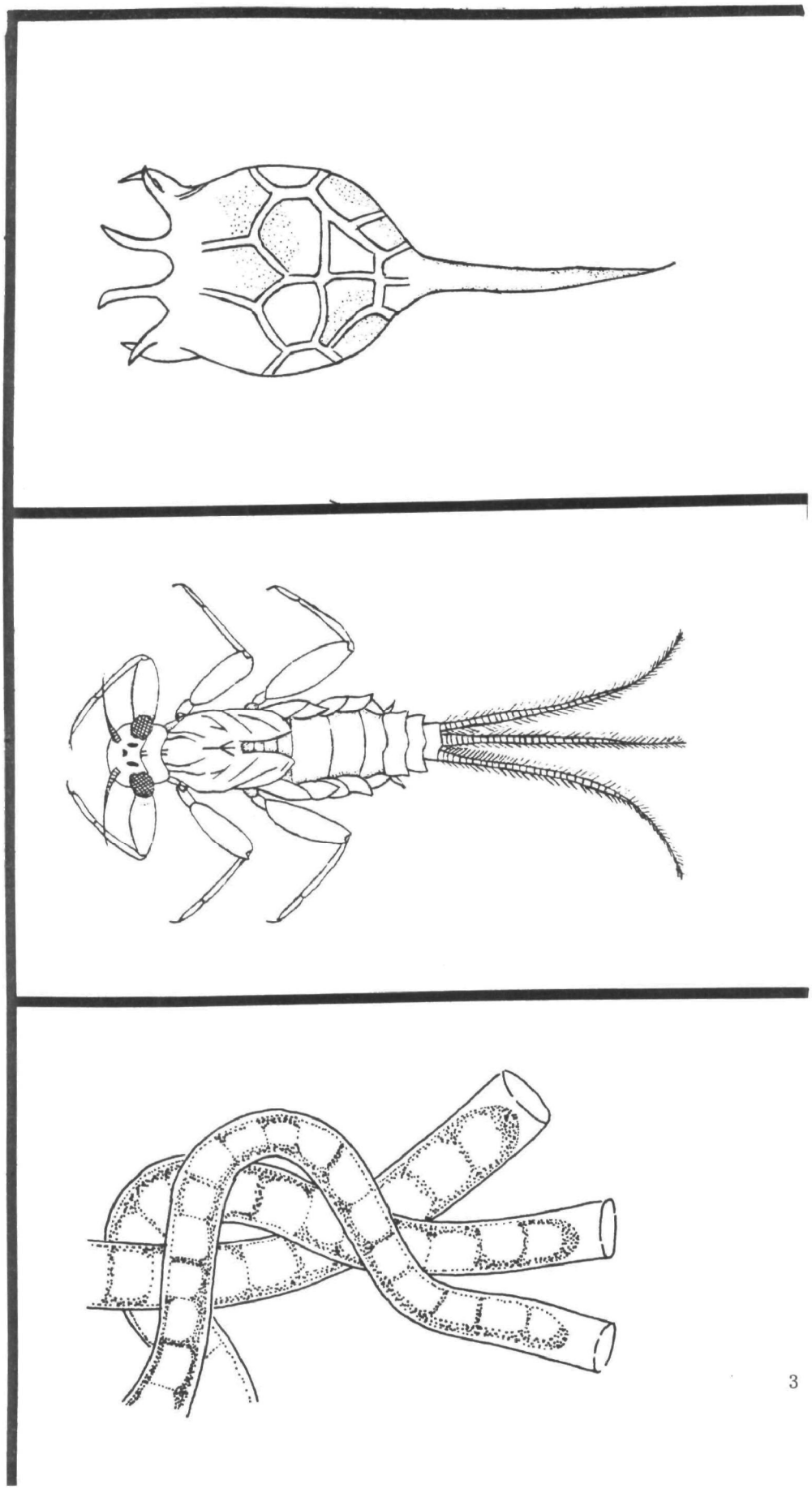
A Assignment of Places and Equipment

B Use of the Microscope

C Use of a Biological Key

These laboratory instructions were prepared by H. W. Jackson, Chief Biologist, and Ralph M. Sinclair, Aquatic Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

Figure 1



Cyanophyta

Hormogonales

Lyngbya

Species of this genus have been found to occur in surface waters, attached to substrates and in some cases associated with pollution. The filament of cells is enclosed by a sheath. Cells range from 4 to 20 microns in diameter and 4 to 10 microns in length.

P. Arthropoda
C. Insecta
O. Ephemeroptera (Mayflies)

Mayflies are common inhabitants of both lakes and streams. They are gill breathers which are usually found crawling about on rocks. Most genera can be distinguished from the stoneflies by the presence of 3 caudal filaments.

P. Rotatoria (Rotifers)
C. Monogononta
F. Brachionidae
G. Keratella

The genus Keratella represents one of the forms of rotifers which are often found in the plankton.

LABORATORY EXERCISES ON TYPES OF ALGAE

I OBJECTIVES

A To learn the terminology and techniques for identifying the major groups of algae.

B To learn to recognize the more common genera on sight.

II MATERIALS

A Compound microscopes equipped with 10, 20, 40, and 100x objectives and mechanical stages.

B Microscope lamps, glass slides, cover glasses, pipettes, algal samples, identification keys, and supplemental books.

III PROCEDURE

A A short preview of the characteristics of the specific group will be presented.

B A representative sample of the algal group under consideration will be distributed. Make a wet mount and focus at 100x on a portion of the algal specimen.

C Using the algae key in your manual follow through the identification with the instructor, making notes and drawings of important characteristics.

D A discussion will accompany and follow the identification pointing out the most important characters and how they were used in the classification.

E This same procedure will be followed with several other algal specimens which illustrate the major morphological forms within a certain group.

F Unknown samples will be distributed for identification by the class.

G A discussion of the classification and importance of the specimen will follow each identification.

This outline was prepared by M.E. Bender, Former Biologist, FWPCA Training Activities, SEC.

LABORATORY: IDENTIFICATION OF DIATOMS

I OBJECTIVES

A To become familiar with important structural features of diatoms.

B To learn to recognize some common forms at sight.

C To learn to identify less common forms using technical keys.

II PROCEDURE

A Transfer a drop of the water sample containing diatoms to a microscope slide. Cover with cover glass and observe under low power (10X) of microscope.

1 Do all of the diatom cells appear to have the same shape? Do some have square ends and some rounded ends? Touch the cover glass with your pencil several times as you observe through the microscope and note the relationship of the two types of ends to one another.

2 Find a place where a round-ended and a square-ended cell are close together and observe these under oil power (90X). The round-ended view is that of the top or bottom of the diatom cell and is called the "valve" view. The square ended view is that of the side of the cell and is called the "girdle" view.

3 In the valve view note the cross lines in the wall. In this diatom there are many fine lines and a smaller number of coarse lines. The former are present in all diatoms and are called "striae" or "striations." The latter are present only in some genera of diatoms and are called "septa," or in other genera, "costae." Which of the two types of lines are continuous from side to side? The space left in the center by the interrupted lines is known as a "false (pseudo) raphe."

4 What is the predominant color of the diatom? How many plastids? In diatoms, the identification is based almost entirely on the characteristics of the cell wall.

5 Make an outline drawing, at least 3 inches long, of a valve view and a girdle view of the diatom. Show the markings in the upper third of each. Label the striae, septa, and false-raphe. Make a drawing of what you image an end view or cross(transverse) section view would be like.

6 Using the key, identify your specimen, listing the alternatives selected.

B Use the key to identify other unknowns as far as possible, listing the alternatives selected in the key. Make a sketch of Navicula and Cyclotella if you identify these forms.

III IMPORTANT TERMS

Capitate - having a known-like end.

Costae - coarse transverse ribs in wall.

False raphe - (see pseudoraphe)

Frustule - the wall of the diatom.

Girdle view - the side view, in which the diatom appears to have square or blunt ends.

Nodule - a lump-like swelling in the center or ends of the valve.

Pseudoraphe - a clear space extending the length of the diatom and bordered on both sides by striae.

Punctae - the dots which comprise the striae.

Raphe - a longitudinal line (cleft) bordered on both sides of striae.

Septa - a self-like partition in the diatom, appearing often as a coarse line.

Striae - fine transverse lines especially evident in the valve view.

Valve view - the top or bottom view, in which the diatom has rounded ends, or is circular in outline.

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This outline was prepared by L.G. Williams, Aquatic Biologist, Formerly with Research and Development, Cincinnati Water Research Laboratory, FWPCA, SEC.

LABORATORY: IDENTIFICATION OF ANIMAL PLANKTON

I INTRODUCTION

A The great majority of organisms commonly encountered in plankton analysis work are plants or at least plant-like (holophytic). From time to time, however, animals appear (holozoic or nonchlorophyll bearing forms), and the ability to recognize them may be quite important.

B Many animals are soft bodied and so are best observed in the living condition, as they shrink and become otherwise distorted on preservation. There are consequently many which will not be available in a suitable form for the following exercise. Only such forms will be dealt with as can readily be obtained alive, or which retain essential characteristics on preservation.

II OBJECTIVES

A To Study the Nature and Use of a Key for Identifying Organisms

B To Introduce the Beginners to the Use of the Microscope

C To Learn to Recognize Basic Animal Types

D To Identify Animal Plankton Species as Available, and to Become Familiar with the Literature

III PROCEDURE

A The Use of the Biological Key

1 Obtain a "Basic Invertebrate Collection" from the instructor.

2 Select a specimen designated by the instructor, and turn to the "Key to Selected Larger Groups of Aquatic Animals "

a Examine your specimen carefully, ' then read the first couplet of statements in the key (1a and 1b).

b Since the specimen is large enough to see, it obviously could not be the object of statement 1a. Therefore due to the nature of the key (as explained in the second paragraph of the introduction) the second alternative (1b) must apply. This alternative instructs us to proceed to couplet 2.

c From here on, follow from couplet to couplet, considering each couplet by itself, until a final selection leads to a name. If this name or couplet is, followed by another couplet number, this means that the group named is further subdivided.

3 Identify the other specimens in the Basic Invertebrate Collection in the same way.

4 Carry the identification further, to genus and species if possible, in one or more of the more detailed keys listed at the end of the "Key to Selected Larger Groups of Aquatic Animals. "

B The Use of the Microscope

1 Obtain preliminary information from the instructor as to how to set up and operate the instrument.

2 Place a prepared slide of a printed letter on the stage and observe it successively under low (100X) and high (45X) powers. When the letter is right side up to you, how does it appear through the microscope?

3 Place a prepared slide of a micro-crustacean on the stage and identify it using the "Key to Selected Larger Groups of Aquatic Animals." Continue your

identification as far as possible using Eddy and Hodson's "Taxonomic Keys."

4 Prepare a "wet mount" under the direction of the instructor and identify the organism. Confirm your identification in one or more of the technical reference books available.

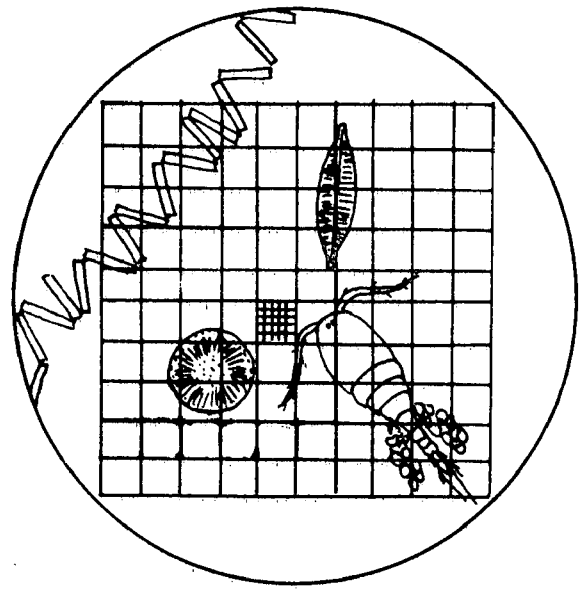
C Identify each of the specimens in the reference collection (labeled "III C") as to phylum and class, and then genus and species if possible (do not spend undue time on the species without assistance).

1 Make a sketch of at least one organism of each phylum observed as an example of a type.

2 Review the collection of "Explanomounts." Sketch and study any with which you are not familiar. Work each one through the keys as if you did not know its name.

D Examine the living material provided. Sketch and identify animal forms encountered as far as time permits. Can you draw any conclusions as to the types of animal life found in the various habitats indicated?

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.



CHAPTER III

TECHNIQUES OF PLANKTON METHODOLOGY

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Calibration and Use of Plankton Counting Equipment	3
Preparation of Permanent Diatom Mounts	4
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TECHNIQUES OF PLANKTON SAMPLING PROGRAMS

I INTRODUCTION

A A planned program of plankton analysis should involve periodic sampling at weekly intervals or more often.

1 Most interference organisms are small, and hence have relatively short-life histories.

2 Populations of such organisms may fluctuate rapidly in response to changing water, weather, or seasons..

3 Seasonal growth patterns of plankton tend to repeat themselves from year to year, thus they are relatively predictable.

B 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.

1 Since the seasons and the years differ, the more records are accumulated, the more useful can they become.

2 As the time for an anticipated bloom of some trouble maker approaches, the frequency of analysis may be increased.

C Detection of a bloom in its early stages will facilitate more economical control.

II FIELD ASPECTS OF THE ANALYSIS PROGRAM

A Two general aspects of sampling are commonly recognized: quantitative and qualitative.

1 Qualitative examination tells <u>what</u> is present.

2 Quantitative tells <u>how much.</u>

3 Either approach is useful, a combination if best.

B Equipment of collecting samples in the field is varied.

1 A half-liter bottle will suffice for surface samples of phytoplankton if carefully taken. If zooplankton also are of interest, 2 or more liters should be collected. (See below).

2 Plankton nets concentrate the sample in the act of collecting, and capture certain larger forms which escape from the bottles. Only the more elaborate types are quantitative however.

3 A kemmerer-type sampler is suggested for depth samples.

4 Other methods such as the Clark-Bumpus sampler or the Juday plankton trap may be employed for special purposes.

C The location of sampling points is important

1 <u>Both</u> shallow and deep samples are suggested.

a "Shallow" samples should be taken at a depth of 6 inches to one foot.

b "Deep" samples should be taken at such intervals as the depth of the reservoir permits. There should be at least one open water sampling point.

c Each major bay or shoal area should have at least one sampling point.

d Additional sampling stations should be established on the basis of experience and resources.

e Samples may be composited if necessary to give an overall summary of conditions. Such summaries are not advised and should be interpreted with care.

A standardized vertical haul however, can be useful for routine comparisons.

III RECORDS

Field conditions greatly affect the plankton, and a record thereof should be carefully identified with the collection.

A Time of day, turbidity of the water, and relative cloudiness affect the amount of light, which affects the vertical distribution of many forms.

B Water temperature affects growth rate and behavior.

C Wind causes water drift, and wave action breaks up colonial forms and disperses accumulations.

D Other conditions may be usefully recorded if circumstances permit.

IV FIELD PRESERVATION OF SAMPLES

Provision should be made for the field stabilization of the sample until the laboratory examination can be made. Techniques and materials are listed below. No "ideal" preservative or technique has yet been developed, each has its virtues.

A Refrigeration or icing. The container containing the sample can be cooled, but under no circumstances should ice be dropped into the sample.

B Preservation by 3-5% formaline is time-tested and widely used. Formaline shrinks animal tissue, fades colors, and makes all forms brittle.

C Ultra-violet sterilization is useful in the laboratory to retard decomposition of plankton.

D Lugol's solution is sometimes useful.

E A special thimerosal preservative was developed by the FWPCA Water Pollution Surveillance System which has proved very satisfactory and is described in reference No. 2.

V SUMMARY AND CONCLUSIONS

A The field sampling program should be carefully planned to evaluate all significant locations in the reservoir or stream, giving due consideration to the capacity of the laboratory.

B Adequate records and notes should be made of field conditions and associated with the laboratory analyses in a permanent file.

C Once a procedure for processing plankton is adopted, it should be used exclusively by all workers at the plant.

D Such a procedure should enable the water plant operator to prevent plankton troubles or at least to anticipate them and have corrective materials or equipment stockpiled.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

PREPARATION AND ENUMERATION OF PLANKTON IN THE LABORATORY

I RECEPTION AND PREPARATION OF SAMPLES

A Preliminary sampling and analysis is an essential preliminary to the establishment of a permanent or semi-permanent program.

B Concentration or sedimentation of preserved samples may precede analysis

1 Batch centrifuge

2 Continuous centrifuge

3 Sedimentation

C Unpreserved (living) samples should be analyzed at once or refrigerated for future analysis.

II PREPARATION OF THIMEROSAL PRESERVATIVE

A The Water Pollution Surveillance System of the FWPCA has developed a modified thimerosal preservative. (Williams, 1962; Weber, 1967) Sufficient stock to make an approximately 3.5% solution in the bottle when filled is placed in the sample bottle in the laboratory. The bottle is then filled with water in the field and returned to the laboratory for analysis.

B Preparation of Thimerosal Preservative

1 Thimerosal is available from many chemical laboratory supply houses; one should specify the water soluble sodium salt.

2 Thimerosal stock: dissolve approximately 1 gram of sodium borate (borax) and approximately 1 gram of thimerosal in 1 liter of distilled water.

The amount of sodium borate and thimerosal may be varied slightly to adjust to different waters, climates, and organic contents.

3 Prepare a saturated aqueous Lugol's solution as follows:

a Add 60 grams of potassium iodide (KI) and 40 grams of iodine to 1 liter of distilled water.

4 Prepare the preservative solution by adding approximately 1.0 ml of the Lugol's solution to 1 liter of thimerosal stock.

III SAMPLE ANALYSIS

A Microscopic examination is most frequently employed in the laboratory to determine what plankton organisms are present and how many there are.

1 Optical equipment need not be elaborate but should include.

a Compound microscope with the following equipment:

1) Mechanical stage
2) Ocular 10X, with Whipple type counting eyepiece or reticule
3) Objectives
approx. 10X(16 mm)
approx. 20X(8 mm)
approx 40X(4 mm)
approx. 95X(1 8 mm)(optional)

A 40X objective with a working distance of 12.8 mm and an erect image may be obtained as special equipment. A water immersion objective (in addition to oil) might be considered for use with water mounts.

Binocular eyepieces are optional

Stage micrometer (this may be borrowed, if necessary, as it is usually used only once, when the equipment is calibrated)

b Inverted microscopes offer certain advantages but are not widely available. The same is true of some of the newer optical systems such as phase contrast microscopy. These are often excellent but expensive for routine plant use.

2 Precision made counting chambers are required for quantitative work with liquid mounts.

a Sedgwick-Rafter cells (hereafter referred to as S-R cell) are used for routine counts of medium and larger forms.

b Extremely small forms or "nanno-plankton" may be counted by use of the nannoplankton (or Palmer) cell, a Fisher-Littman cell, a hemacytometer, the Lackey drop method, or by use of an inverted microscope.

3 Previous to starting serious analytical work, the microscope should be calibrated as described elsewhere Dimensions of the S-R cell should also be checked, especially the depth

4 Automatic particle counters may be useful for coccoid organisms.

B Quantitative Plankton Counts

1 All quantitative counting techniques involve the filling of a standard cell of known dimensions with either straight sample or a concentrate or dilution thereof.

2 The organisms in a predetermined number of microscope fields or other known area are then observed, and by means of a suitable series of multiplier factors, projected to a number or quantity per ml gallon, etc.

3 Direct counting of the unconcentrated sample eliminates manipulation, saves time, and reduces error If frequency of organisms is low, more area may need to be examined or concentration of the sample may be in order.

4 Conventional techniques employing concentration of the sample provide more organisms for observation, but because they involve more manipulations, introduce additional errors and take more time

C Several methods of counting plankton are in general use.

1 The numerical or clump count is regarded as the simplest

a Every organism observed must be enumerated. If it cannot be identified, assign a symbol or number and make a sketch of it on the back of the record sheet.

b Filaments, colonies and other associations of cells are counted as units, equal to single isolated cells Their identity as indicated on the record sheet is the key to the significance of such a count

2 Individual cell count In this method, every cell of every colony or clump of organisms is counted, as well as each individual single-celled organism.

3 The areal standard unit method offers certain technical advantages, but also involves certain inherrent difficulties.

a An areal standard unit is 400 square microns. This is the area of one of the smallest subdivided squares

in the center of the Whipple eyepiece at a magnification of 100X

b In operation, the number of areal units of each species is recorded on the record sheet rather than the number of individuals Average areas of the common species are sometimes printed on record sheets for a particular plant to obviate the necessity of estimating the area of each cell observed individually

c The advantage of the method lies in the cognizance taken of the relative masses of the various species as indicated by the area presented to the viewer These areas, however, are often very difficult to estimate.

4 The cubic standard unit method is a logical extension of the areal method, but has achieved less acceptance.

5 Separate field count

a In counting separate fields, the question always arises as to how to count organisms touching or crossed by the edge of the Whipple field Some workers estimate the proportion of the organism lying inside the field as compared to that outside. Only those which are over half way inside are counted.

b Another system is to select two adjacent sides of the square for reference, such as the top and left boundaries. Organisms touching these lines in any degree, from outside or inside, are then counted, while organisms touching the opposite sides are ignored. It is important to adopt some such system and adhere to it consistently

c It is suggested that if separate microscopic fields are examined, a standard number of ten be adopted. These should be evenly spaced in two rows about one third of the distance down from the top and one third of the distance up from the bottom of the S-R cell.

6 Multiple area count. This is an extension of the separate field count A considerable increase in accuracy has recently been shown to accrue by emptying and refilling the S-R cell, after each group of fields are counted and making up to 5 additional such counts. This may not be practical with high counts.

7 The strip count. When a rectangular slide such as the S-R cell is used, a strip (or strips) the entire length (or known portion thereof) of the cell may be counted instead of separate isolated fields. Marking the bottom of the cell by evenly spaced cross lines as explained elsewhere greatly facilitates counting.

a When the count obtained is multiplied by the ratio of the width of the strip counted to the width of the cell, the product is the estimated number of organisms in the cell, or per ml

b When the material in the cell is unconcentrated sample water, this count represents the condition of the water being evaluated without further calculation

8 Once a procedure for concentration and/or counting is adopted by a plant or other organization it should be used consistently from then on so that results from year to year can be compared.

D Differential or qualitative "counts" are essentially lists of the kinds of organisms found.

E Proportional or relative counts of special groups are often very useful. For example, diatoms. It is best to always count a standard number of cells.

F Plankton are sometimes measured by means other than microscopic counts

1 Settled volume of killed plankton in an Imhoff cone may be observed after a standard length of time This will evaluate primarily only the larger forms.

2 A gravimetric method employs drying at 60°C for 24 hours followed by ashing at 600°C for 30 minutes. This is particularly useful for chemical and radiochemical analysis

3 A method for chlorophyll extraction involves filtration, drying for 24 hours, extraction with methyl alcohol, and evaluation in a colorimeter or acetone extracts, using a spectrophotometer This is widely used in "productivity" studies.

4 The membrane (molecular) filter has a great potential, but a generally acceptable technique has yet to be perfected.

a Bacteriological techniques for coliform determination are widely accepted.

b Nematodes and larger organisms can readily be washed off of the membrane after filtration

c It is also being used to measure ultraplankton that pass treatment plant operations.

d Membranes can be cleared and organisms deposited thereon observed directly, although accessory staining is desirable.

e Difficulties include a predilection of extremely fine membranes to clog rapidly with silt or increase in plankton counts, and the difficulty of making observations on individual cells when the organisms are piled on top of each other It is sometimes necessary to dilute a sample to obtain suitable distribution

IV SUMMARY AND CONCLUSIONS

A The field sampling program should be carefully planned to evaluate all significant locations in the reservoir or stream, giving due consideration to the capacity of the laboratory

B Adequate records and notes should be made of field conditions and associated with the laboratory analyses in a permanent file.

C Optical equipment in the laboratory should be calibrated.

D Once a procedure for processing plankton is adopted, it should be used exclusively by all workers at the plant

E Such a procedure should enable the water plant operator to prevent plankton troubles or at least to anticipate them and have corrective materials or equipment stockpiled.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

CALIBRATION AND USE OF PLANKTON COUNTING EQUIPMENT

I INTRODUCTION

A With the exception of factory-set instruments, no two microscopes can be counted upon to provide exactly the same magnification with any given combination of oculars and objectives. For accurate quantitative studies, it is therefore necessary to standardize or "calibrate" each instrument against a known standard scale. One scale frequently used is a microscope slide on which two millimeters are subdivided into tenths, and two additional tenths are subdivided into hundredths. Figure 3.

B In order to provide an accurate measuring device in the microscope, a Whipple Plankton Counting Square or reticule (Figure 2a) is installed in one ocular (there are many different types of reticules). This square is theoretically of such a size that with a 10X objective, a 10X ocular, and a tube length of 160 mm, the image of the square covers a square area on the slide one mm on a slide. Since this objective is rarely attained however, most microscopes must be standardized or "calibrated" as described below in order to ascertain the actual size of the Whipple Square as seen through the microscope (hereinafter referred to as the "Whipple field"). This process is schematically represented in Figures 5 and 7. If the Whipple eyepiece is to be used at more than one magnification, it must be recalibrated for each. A basic type of monocular microscope is shown in Figure 1.

C Microscopes with two eyepieces (binocular) are a convenience but not essential. Like modern cars they are not only great "performers," but also complicated to service or, in this instance, calibrate. On some instruments, changing the interpupillary distance also changes the tube length, on others it does not. The "zoom" feature on certain scopes is also essentially a system for changing the tube length.

The resultant is that in addition to calibration at each combination of eyepiece and objective, any other factor which may affect magnification must also be considered. In some instances this may mean setting up a table of calibrations at a series of microscope settings.

Another procedure is to select a value for each of the variables involved (interpupillary distance, zoom, etc.) and calibrate the scope at that combination. Then each time the scope is to be used for quantitative work, re-set each variable to the value selected. A separate multiplication factor must be calculated for each adjustment which changes the magnification of the instrument.

Since the Whipple Square can be used to measure both linear dimensions and square areas, both should be recorded on an appropriate form. A suggested format is shown in Figure 6.

(Data written in are used as an illustration and are not intended to apply to any particular microscope. An unused form is included as Figure 6-A.)

II THE CALIBRATION PROCEDURE

A Installing the Whipple Square or Reticule

To install the reticule in the ocular (usually the right one on a binocular microscope), carefully unscrew the upper lens mounting and place the reticule on the circular diaphragm or shelf which will be found approximately half way down inside (Figure 4). Replace the lens mounting and observe the markings on the reticule. If they are not in sharp focus, remove and turn the reticule over.

On reticules with the markings etched on one side of a glass disc, the etched surface can usually be recognized by shining the disc at the proper angle in a light. The markings will usually be in the best focus with the etched surface down. If the markings are sandwiched between two glass discs cemented together, both sides are alike, and the focus may not be quite as sharp.

B Observation of the Stage Micrometer

Replace the ocular in the microscope and observe the stage micrometer as is illustrated schematically in Figure 5: Calibration of the Whipple Square. On a suitably ruled form such as the one illustrated, Figure 6, Calibration Data, record the actual distance in millimeters subtended by the image of

the entire Whipple field and also by each of its subdivisions. This should be determined for each significant settling of the interpupillary distance for a binocular microscope, and also for each combination of lenses employed. Since oculars and objectives marked with identical magnification, and since microscope frames too may differ, the serial or other identifying number of those actually calibrated should be recorded. It is thus apparent that the determinations recorded will only be valid when used with the lenses listed and on that particular microscope.

C Use of the 20X Objective

Due to the short working distance beneath a 46X (4mm) objective, it is impossible to focus to the bottom of the Sedgewick-Rafter plankton counting cell with this lens. A 10X (16mm) lens on the other hand "wastes" space between the front of the lens and the coverglass, even when focused on the bottom of the cell. In order to make the most efficient use possible of this cell then, an objective of intermediate focal length is desirable. A lens with a focal length of approximately 8 mm, having a magnification of 20 or 21X will meet these requirements. Such lenses are available from American manufacturers and are recommended for this type of work.

III CHECKING THE CELL

The internal dimensions of a Sedgewick-Rafter plankton counting cell should be 50 mm long by 20 mm wide by 1 mm deep (Figure 8).

The actual horizontal dimensions of each new cell should be checked with calipers, and the depth of the cell checked at several points around the edge using the vertical focusing scale engraved on the fine adjustment knob of most microscopes. One complete rotation of the knob usually raises or lowers the objective 1 mm or 100 microns (and each single mark equals 1 micron). Thus, approximately ten turns of the fine adjustment knob should raise the focus from the bottom of the cell to the underside of a coverglass resting on the rim. Make these measurements on an empty cell. The use of a No. 1 or 1-1/2, 24 × 60 mm coverglass is recommended rather than the heavy coverglass that comes with the S-R cell, as the thinner glass will somewhat conform to any irregularities of the cell rim (hence, also making a tighter seal and reducing evaporation when in actual use). Do not attempt to focus on the upper surface of the rim of an empty cell for the above depth measurements, as the coverglass is supported by the highest points of the rim only, which are very difficult to identify. Use the average of all depth measurements as the "true" depth of the cell. To simplify calculations below, it will be assumed that we are dealing with a cell with an average depth of exactly 1.0 mm.

IV PROCEDURE FOR STRIP COUNTS USING THE SEDGEWICK-RAFTER CELL

A Principles

Since the total area of the cell is 1000 mm^2, the total volume is 1000 mm^3 or 1 ml. A "strip" the length of the cell thus constitutes a volume (V_1) 50 mm long, 1 mm deep, and the width of the Whipple field.

The volume of such a strip in mm^3 is:

$$V_1 = 50 \times \text{width of field} \times \text{depth}$$

$$= 50 \times w \times 1$$

$$= 50\ w$$

In the example given below on the plate entitled Calibration Data, at a magnification of approximately 200X with an interpupillary setting of "60", the width of the Whipple field is recorded as approximately 0.55 mm (or 550 microns). In this case, the volume of the strip is:

$$V_1 = 50\ w = 50 \times 0.55 = 27.5\ (mm^3)$$

B Calculation of Multiplier Factor

In order to convert plankton counts per strip to counts per ml, it is simply necessary to multiply the count obtained by a factor (F_1) which represents the number of times the volume of the strip examined (V_1) would be contained in 1 ml or 1000 mm^3. Thus in the example given above:

$$F_1 = \frac{\text{volume of cell in } mm^3}{\text{volume examined in } mm^3}$$

$$= \frac{1000}{V} = \frac{1000}{27.5} = 36.36$$

$$= \text{approx. } 36$$

If more than one strip is to be counted, the factor for two, three, etc., strips could be calculated separately using the same relationships outlined above, changing only the measurement for the length of

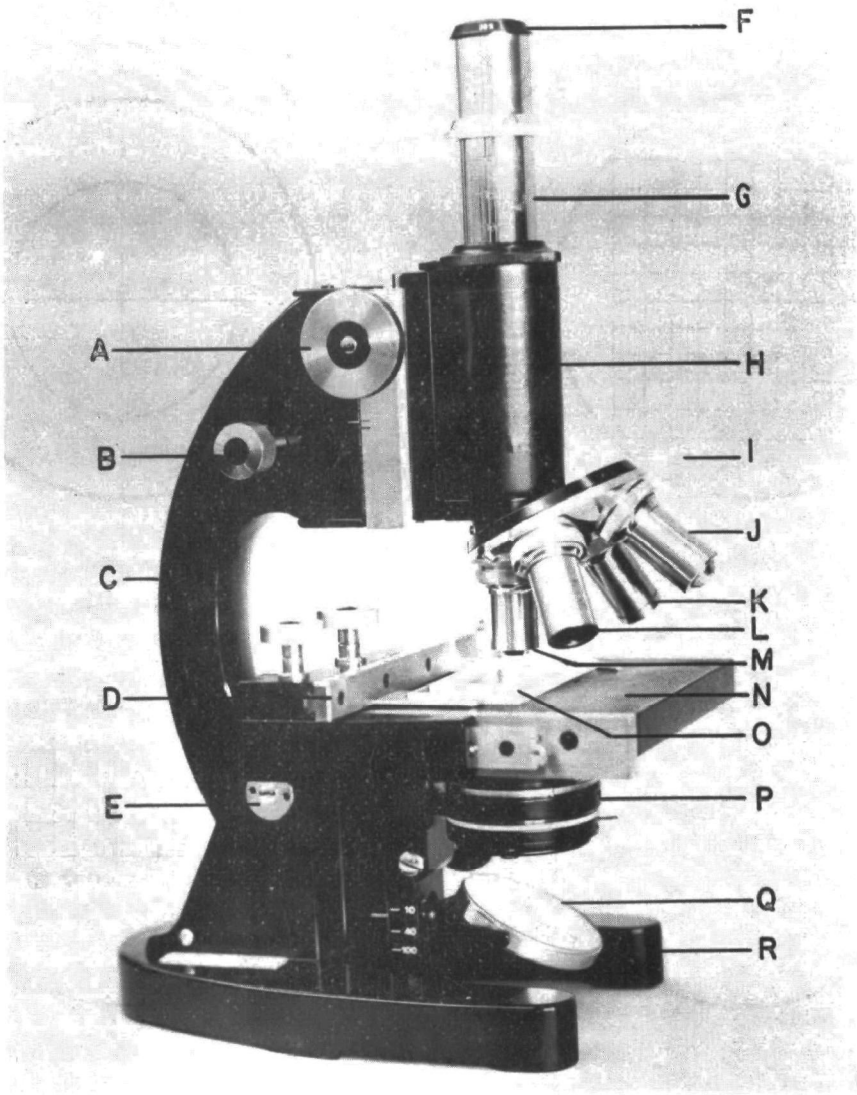


Figure 1. THE COMPOUND MICROSCOPE

A) coarse adjustment; B) fine adjustment; C) arm or pillar; D) mechanical stage which holds slides and is movable in two directions by means of the two knobs; E) pivot or joint. This should not be used or "broken" while counting plankton; F) eyepiece (or ocular cf: figure 4); G) draw tube. This will be found on monocular microscopes only (those having only one eyepiece). Adjustment of this tube is very helpful in calibrating the microscope for quantitative counting (Sec. 5.5.2.2.). H) body tube. In some makes of microscopes this can be replaced with a body tube having two eyepieces, thus making the 'scope into a "binocular." I) revolving nosepiece on which the objectives are mounted; J) through M are objectives, any one of which can be turned toward the object being studied. In this case J is a 40X, K is a 100X, L is a 20X, and M is a 10X objective. The product of the magnification power of the objective being used times the magnification power of the eyepiece gives the total magnification of the microscope. Different makes of microscopes employ objectives of slightly different powers, but all are approximately equivalent. N) stage of the microscope; O) Sedgwick-Rafter cell in place for observation; P) substage condenser; Q) mirror; R) base or stand; note: for information on the optical system, consult reference 3. (Photo by Don Moran.).

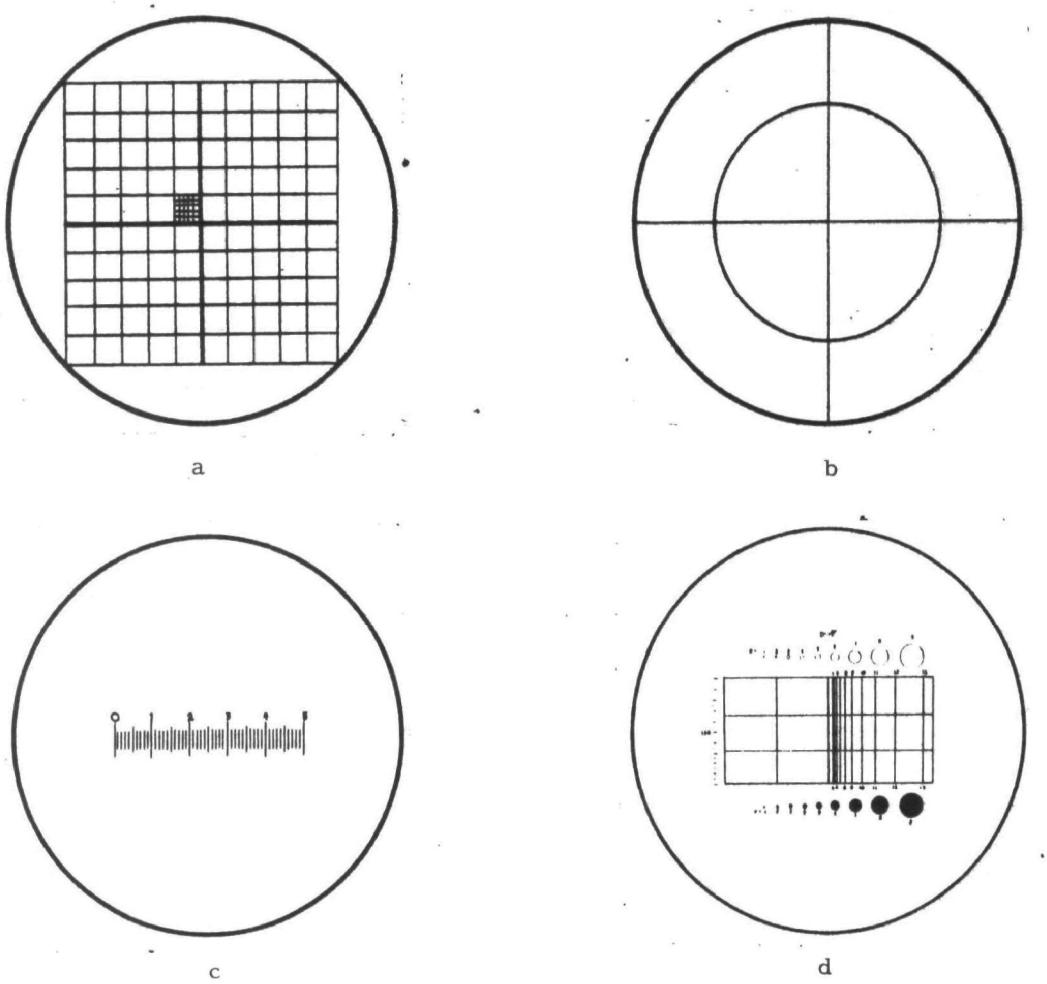


Figure 2

Types of eyepiece micrometer discs or reticules (reticules, graticules, etc.). When dimensions are mentioned in the following description, they refer to the markings on the reticule discs and not to the measurements subtended on the microscope slide. The latter must be determined by calibration procedures such as those described elsewhere. (a) Whipple plankton counting eyepiece. The fine rulings in the subdivided square are sometimes extended to the margin of the large square to facilitate the estimation of sizes of organisms in different parts of the field. (b) Quadrant ruling with 8.0 mm circle, for counting bacteria in milk smears for example. (c) Linear scale 5.0 mm divided into tenths. For measurement of linear dimensions. (d) Porton reticule for estimating the size of particles. The sizes of the series of discs is based on the square root of two so that the areas of successive discs double as they progress in size.

strip counted. Thus for two strips in the example cited above:

$$V_2 = 100W = 100 \times 0.55 = 55 \text{ mm}^3$$

$$F_2 = \frac{1000}{V_1} = \frac{1000}{55} = 18.2$$

It will however be noted that $F_2 = \frac{F_1}{2}$.

Likewise a factor F_3 for three strips would equal $\frac{F_1}{3}$ or approximately 12, etc.

C An Empirical "Step-Off" Method

A simpler but more empirical procedure for determining the factor is to consider that if a strip 20 mm wide were to be counted the length of the cell, that the entire 1000 mm^3 would be included since the cell is 20 mm wide and 1 mm deep.

This 20 mm strip width can be equated to 1000 mm^3. If a strip (or the total of 2 or more strips) is less than 20 mm in width, the quotient of 20 divided by this width will be a multiplier factor for converting from count per strip(s) to count per ml.

Thus in the example cited above where at an approximate magnification of 200X and with an interpupillary setting of 60, the width of the Whipple field is .55 mm. Then:

$$F_1 = \frac{20}{.55} = 36.36 \text{ or approx. } 36$$

(as above)

If two strips are counted:

$$\begin{array}{r} .55 \\ +\ .55 \\ \hline 1.10 \end{array} \text{ and } F_2 = \frac{20}{1.1} = 18.2 = \text{approx. } 18, \text{etc.}$$

This same value could be obtained without the use of a stage micrometer by carefully moving the cell sidewise across the field of vision by the use of a mechanical stage. Count the number of Whipple fields in the width of the cell. There should be approximately 18 in the instance cited above.

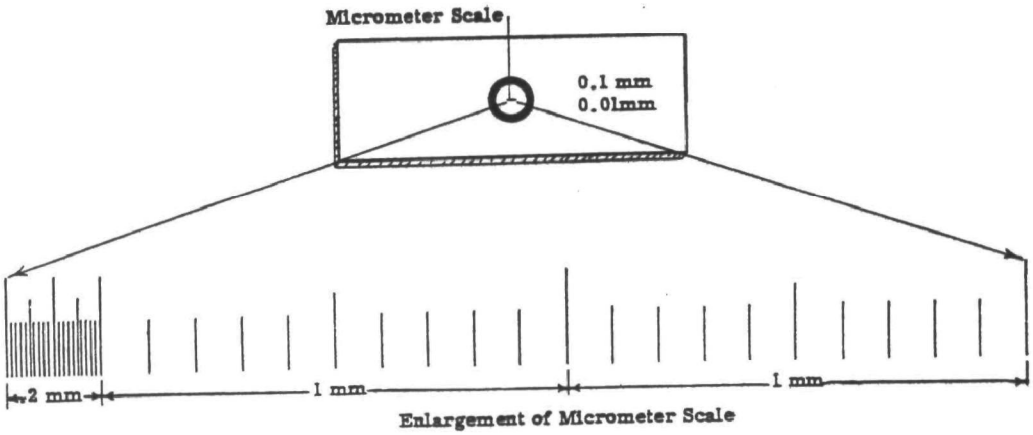
V SEPARATE FIELD COUNT USING THE SEDGEWICK-RAFTER CELL

A Circumstances of Use

The use of concentrated samples, local established programs, or other circumstances

Figure 3. STAGE MICROMETER

The type illustrated has two millimeters divided into tenths, plus two additional tenths subdivided into hundredths.



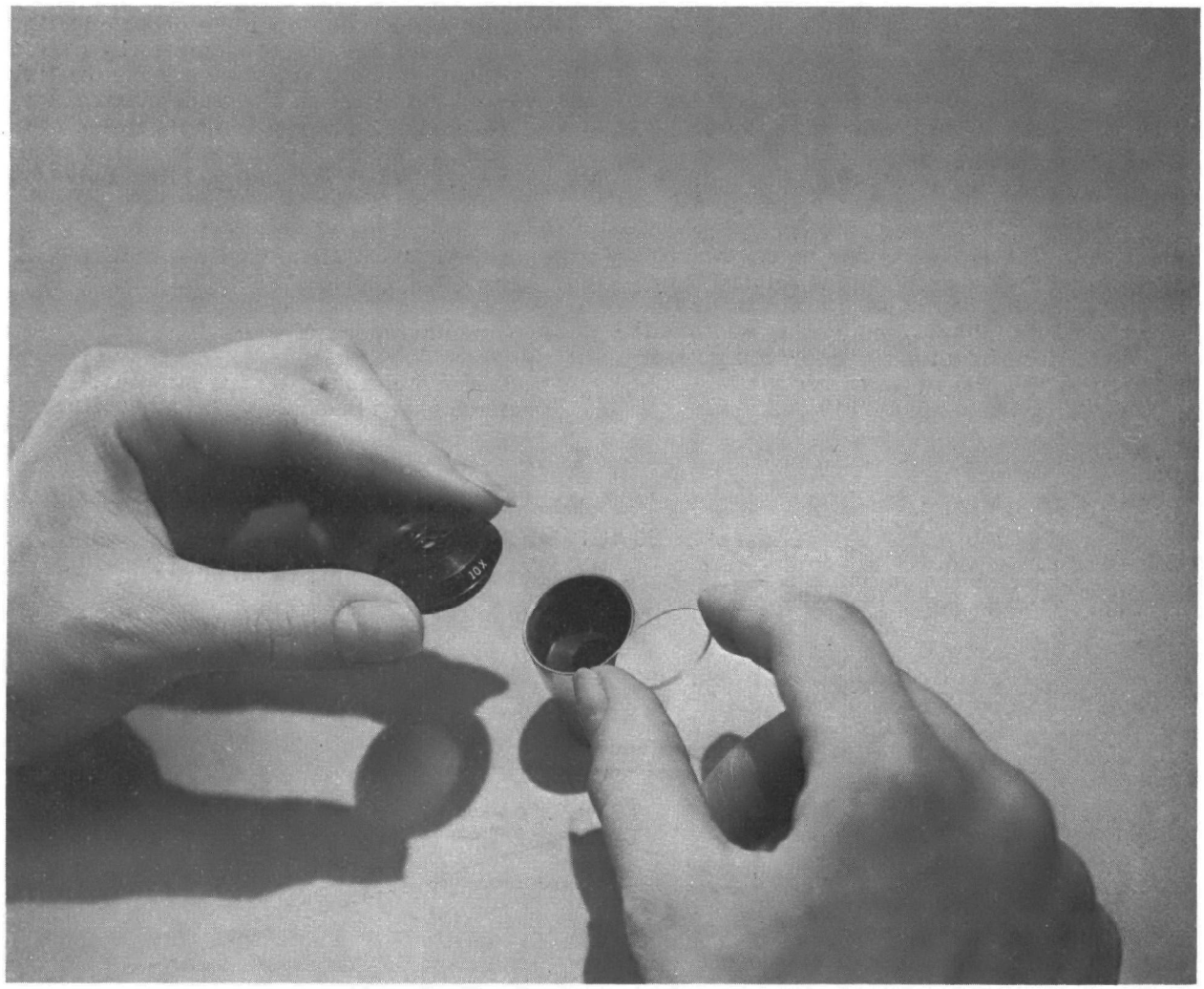


Figure 4. Method of Mounting the Whipple Disc in an Ocular. Note the upper lens of the ocular which has been carefully unscrewed, held in the left hand, and the Whipple disc, held in the right hand. (Photo by Don Moran).

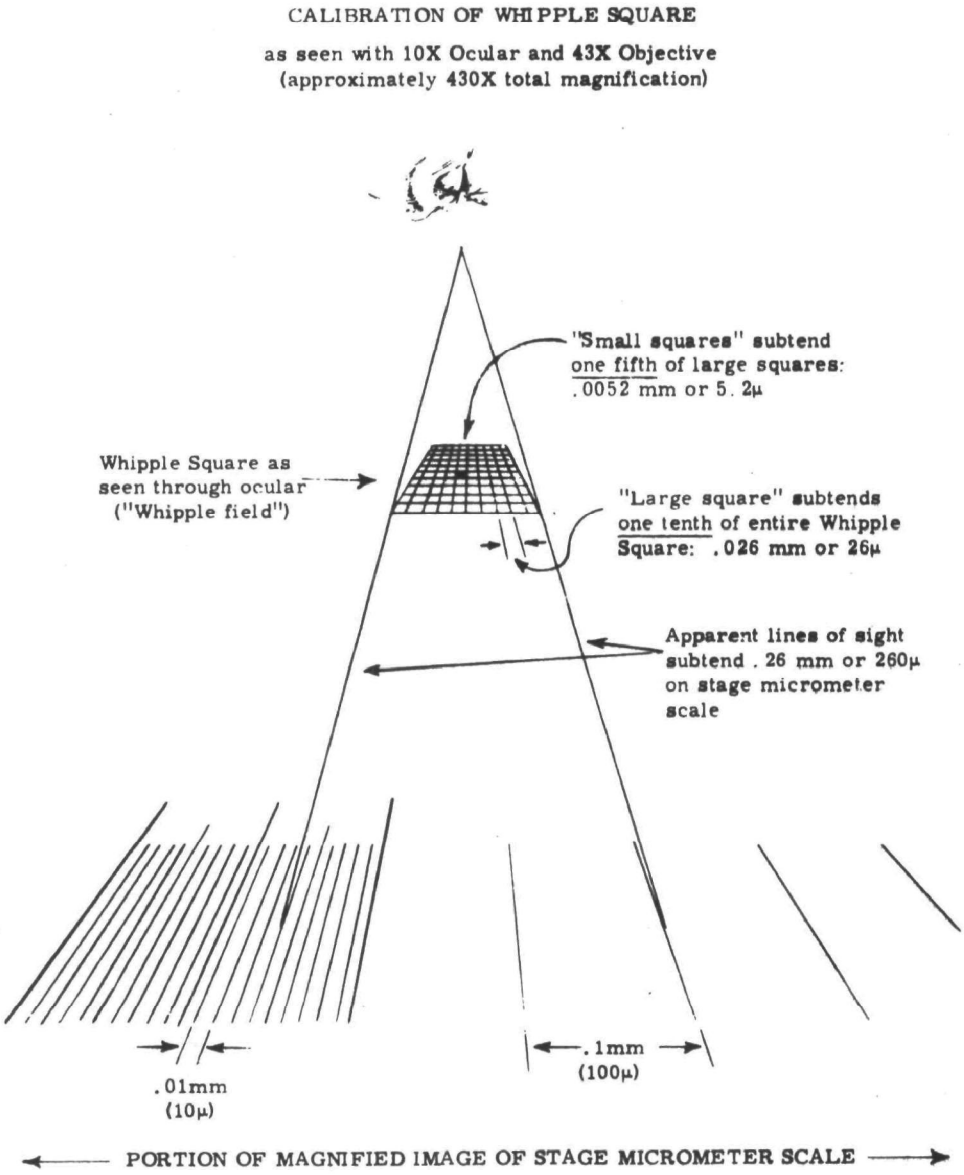


Figure 5

CALIBRATION OF THE WHIPPLE SQUARE

The apparent relationship of the Whipple Square is shown as it is viewed through a microscope while looking at a stage micrometer with a magnification of approximately 430X (10X ocular and 43X objective).

MICROSCOPE CALIBRATION DATA

Microscope No 62379

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
476421(10X)	50	1.130	0.113	0.0226	8.9
and Ocular Serial No	60	1.115	0.111	0.0222	9.0
1296742(10X)	70	1.100	0.110	0.0222	9.1
200X, obtained with					**(2 S-R Strips)**
Objective Serial No.					
6149289(21X)	50	0.560	0.056	0.0112	17.9
and Ocular Serial No.	60	0.550	0.055	0.0110	18.2
1296742(10X)	70	0.545	0.054	0.0109	18.3
400X, obtained with					**(Nannoplankton) (cell-20 fields)**
Objective Serial No.					
2391841(43X)	50	0.267	0.0267	.0053	1724.
and Ocular Serial No.	60	0.263	0.0263	.0053	1786.
1296742(10X)	70	0.260	0.0260	.0052	1852.

*1 mm = 1000 microns

Microscope calibration data. The form shown is suggested for the recording of data pertaining to a particular microscope. Headings could be modified to suit local situations. For example, "Interpupillary Setting" could be replaced by "Tube Length" or the "2S-R Strips" could be replaced by "per field" or "per 10 fields."

Figure 6

MICROSCOPE CALIBRATION DATA

Microscope No. _____

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with _____ and Ocular Serial No _____ Objective Serial No. _____					(2 S-R Strips)
200X, obtained with _____ and Ocular Serial No. _____ Objective Serial No. _____					(2 S-R Strips)
400X, obtained with _____ and Ocular Serial No. _____ Objective Serial No. _____					(Nannoplankton) (cell-20 fields)

*1mm = 1000 microns

BI. AQ. pl 8. 10. 60.

Figure 6-A

MICROSCOPE CALIBRATION DATA

Suggested work sheet for the calibration of a microscope. Details will need to be adapted to the particular instrument and situation.

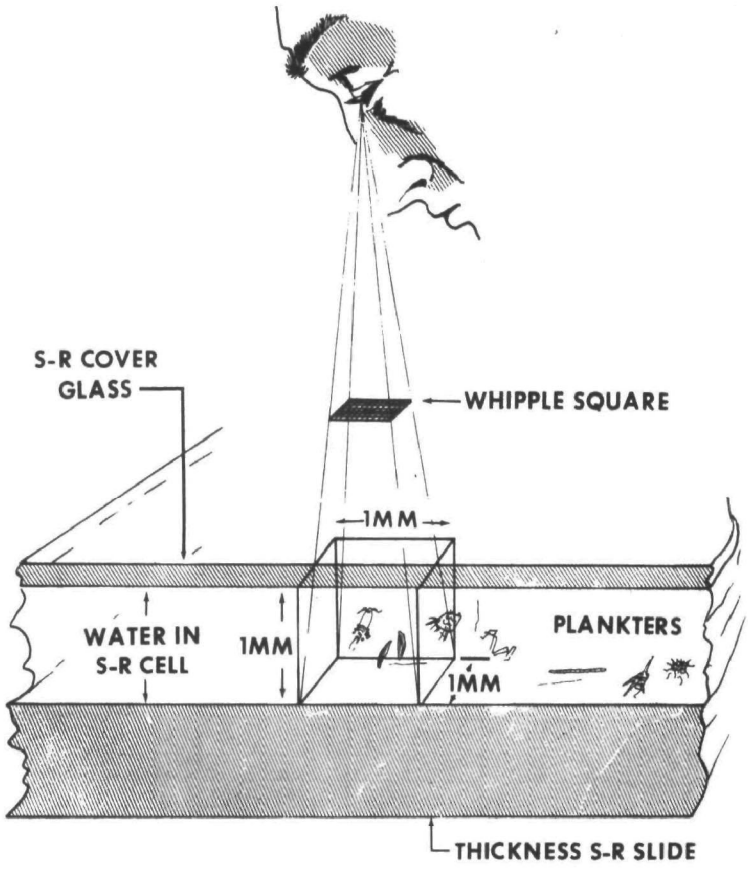


Figure 7

A cube of water as seen through a Whipple square at 100X magnification in a Sedgewick-Rafter cell. The figure is drawn as if the microscope were focused on the bottom of the cell, making visible only those organisms lying on the bottom of the cell. The little "bug" (copepod) halfway up, and the algae filament at the top would be out of focus. The focus must be moved up and down in order to study (or count) the entire cube.

may make it necessary to employ the more conventional technique of counting one or more separate Whipple fields instead of the strip count method. The basic relationships outlined above still hold, namely:

$$F = \frac{\text{volume cell in mm}^3}{\text{volume examined in mm}^3}$$

B Principles Involved

The volume examined in this case will consist of one or more squares the dimensions of the Whipple field in area and 1 mm in depth (Figure 7). Common practice for routine work is to examine 10 fields, but exceptionally high or low counts or other circumstances may indicate that some other number of fields should be employed. In this case a "per field" factor may be determined to be subsequently divided by the number of fields examined as with the strip count. The following description however is based on an assumed count of 10 fields.

C Calculation of Multiplier Factor

As stated above, the total volume represented in the fields examined consists of the total area of the Whipple fields multiplied by the depth.

V_4 = (side of Whipple field)^2 × depth

(1 mm) × no. of fields counted)

For example, let us assume an approximate magnification of 100X (see Figures 6 and 7 and an interpupillary setting of "50". The observed length of one side of the Whipple field in this case is 1.13 mm. The calculation of V_4 is thus:

V_4 = side^2 × depth × no. of fields

= 1.13 × 1.13 × 1 × 10 = 12.8 mm^3

The multiplier factor is obtained as above (Section IV A):

$$F_4 = \frac{\text{volume cell in mm}^3}{\text{volume examined in mm}^3}$$

$$= \frac{1000}{12.8} = \text{(approx.) } 78$$

(If one field were counted, the factor would be 781, for 100 fields it would be 7.8.)

NANNOPLANKTON COUNTING

For counting nannoplankton using the high dry power (10X ocular and 43X objective) and the "nannoplankton counting cell" (Figure 9) which is 0.4 mm deep, a minimum of 20 separate Whipple fields is suggested. The same general relationships presented above (Section IV) can be used to obtain a multiplier or factor (F_5) to convert counts per 20 fields to counts per ml.

To take another example from Figure 4, at an approximate magnification of 400X and an interpupillary setting of 70 (see also Figure 3) we observe that one side of the Whipple field measures 0.260 mm. The volume of the fields examined is thus obtained as follows:

V_5 = side^2 × depth × no. of fields

= 0.26 × 0.26 × 0.4 × 20 = .54 mm^3

and $F_5 = \frac{1000}{.54}$ = (approx.) 1850

It should be noted that the volume of the nannoplankton cell, .1 ml, is of no significance in this particular calculation.

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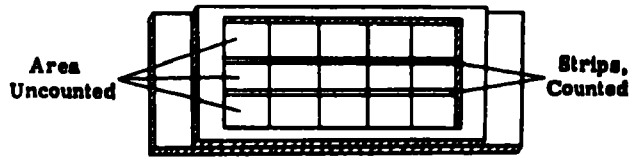

Figure 8

Sedgewick-Rafter counting cell showing bottom scored across for ease in counting strips. The "strips" as shown in the illustration simply represent the area counted, and are not marked on the slide. The conventional dimensions are 50 X 20 X 1 mm, but these should be checked for accurate work.

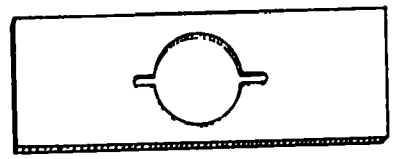

Figure 9

Nannoplankton cell. Dimensions of the circular part of the cell are 17.9 mm diameter X 0.4 mm depth. When covered with a coverglass, the volume contained is 0.1 ml. The channels for the introduction of sample and the release of air are 2 mm wide and approximately 5 mm long. This slide is designed to be used with the 4 mm or 43X (high dry) objective.

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This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, FWQA, Cincinnati, OH 45226.

PREPARATION OF PERMANENT DIATOM MOUNTS

I The identification of many diatoms to genus and all diatoms to species requires that the cells be free of organic contents. This is necessary because the taxonomy of the diatoms is based on the structure of the frustule (shells) of the organisms and many features are masked by the presence of organic materials which may remain inside. It is also necessary that at least 1000X magnification (oil immersion) be used to detect the structural features used in identification. No simple procedure for the accurate routine counting of diatoms has yet been developed.

II MATERIALS NECESSARY

A Sample Concentration

1 Centrifuge (such as Universal DU)

2 100 ml centrifuge tubes

3 Membrane filter apparatus

4 Vacuum

B Slide Preparation

1 Slides, 1 X 3 inch, frosted-end

2 Cover glasses, circular #1, 18 mm, 0.13 - .16 mm thick

3 Resinous mounting medium (such as Harleco microscope mounting medium)

4 Hot plates

a 180° F

b 700° F

5 Disposable pipettes

6 3 X 6 X 1/4 inch steel plate

III PROCEDURE

A The volume of sample needed will vary according to the density of diatoms and silt, and only with experience can the correct sample size be determined. In most cases, 100 ml will be sufficient.

1 Spin 100 ml at 1000 G for 20 minutes.

2 Withdraw the supernatant liquid with an aspirator, being careful not to disturb the concentrate at the bottom of the centrifuge tube. (Draw off all but 2-3 ml.)

3 Transfer the concentrate to a labelled 10 ml disposable vial. Label the vial with a magic marker, diamond pencil, or "time" label.

4 If the sample has been preserved with formalin, or contains more than 1.0 gram per liter dissolved solids, it will be necessary to wash the concentrate with distilled water. In this case, transfer the entire concentrate to a 15 ml centrifuge tube. Dilute to 15 ml with distilled water, making certain that the sample is well mixed. Spin for 10 minutes at full speed in a clinical centrifuge. Withdraw the supernatant liquid, and refill with distilled water. Spin again for 10 minutes. Withdraw the supernatant liquid as before, return the concentrate to the rinsed vial in 2-3 ml of distilled water and proceed with the mounting.

5 If more than 200 ml of sample must be centrifuged to obtain sufficient material to prepare a diatom slide, concentrate the diatoms by filtering the sample through a 1.2 micron pore diameter membrane filter. Transfer the filter to a 15 ml centrifuge tube, and dissolve with 90% acetone. Centrifuge 10 minutes (full speed) and decant with an aspirator. Refill with 90% acetone,

agitate, and spin again for 10 minutes. Repeat until three fresh acetone washes have been used. Replace the acetone with 2-3 ml of distilled water and transfer to a labelled vial as described in #4.

B If the loss of minute forms in supernatant is suspected, spin 100 ml at 1000 gs in a batch centrifuge for as long as may be necessary, then proceed as below.

C Mounting

1 Heat the hot plates to the prescribed temperatures.

2 Place one cover glass on the steel plate for each sample.

3 Place the steel plate on the 180° F hot plate.

4 Transfer a drop of sample to a cover glass.

5 Allow the water to evaporate (caution: do not allow it to boil.)

6 Continue to add more sample until a thin layer of material is noticeable on the dry cover glass, or until all of the concentrate has been used. This step is especially critical, and can be learned only by trial and error.

7 Transfer the steel plate to the 700° F hot plate for 20-30 minutes. (The plate should be hot enough to incinerate paper.)

8 While the material is on the high temperature hot plate, label the microscope slides (use a #2 pencil or a fine point drawing pen); place them on the low temperature hot plate, which now has been reset to approximately 275° F.

9 Place a drop of mounting resin on the microscope slides and allow the solvent to evaporate.

10 When the incineration of the material on the cover glasses is complete, transfer the cover glasses, while still hot, to the mounting medium.

11 Allow the resin to penetrate the frustules (1-2 minutes).

12 Remove the slide, place it on a cool desk top, and press the cover glass lightly with a pencil eraser for a few seconds. The medium will harden in 5-10 seconds.

13 Scrape off the excess resin with a razor blade.

D The preparation is now ready for examination under an oil immersion objective.

ACKNOWLEDGEMENT:

Certain portions of this outline contains training material from a prior outline by M. E. Bender.

This outline was prepared by Dr. C.I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

DETERMINATION OF ODORS

I INTRODUCTION

Odor shall be determined substantially as prescribed by the 11th edition of "Standard Methods for the Examination of Water and Sewage", subject to certain stipulations and modifications made necessary by the International Joint Commission.

The procedure and technique to be followed are described below.

II REAGENTS AND APPARATUS

A Odor-free water - prepared by passing tap water through activated carbon at a slow rate of speed. Activated carbon can be placed at the bottom of a 20-liter glass bottle. The bottle can be connected to the tap by rubber tubing leading to glass tubing above the water. The outlet from the bottom of the bottle should be glass tubing. A trap made of inch glass tubing filled with activated carbon is placed at the end of the outlet.

B 500 ml glass-stoppered Erlenmeyer flasks, each flask with a number. Glassware must be thoroughly cleaned and rinsed several times with odor-free water before each use.

C Chemical Thermometer (0-100°C)

D 10 ml Mohr pipettes, 25 ml graduated cylinders, 50 ml graduated cylinders, 200 ml graduated cylinders, 500 ml graduated cylinders. Other pipettes and cylinders as needed.

E One liter glass-stoppered bottles to hold samples of water being examined. Other glass bottles and flasks as needed.

III PRECAUTIONS

A Certain conditions are required to obtain consistent results. Considerable practice with the test is desirable to develop consistent sensitivity to the sense of smell.

1 In view of the perishability of the odor test, these determinations should be made immediately after collection.

2 The prepared odor-free water should be truly free of all detectable odor.

3 All glassware must be free of odor. This is accomplished by thorough cleansing followed by several rinses with odor-free water.

4 All dilutions should be compared with an odorless standard. This aids the observer in deciding whether air odor is present or not.

5 All dilutions when examined for odor should be of a uniform temperature, deviation not to exceed 1°C.

6 A sudden change in the character of the odor during the testing procedure should be considered as a warning that there may be interference from outside odors or that the diluting water may not be odor-free. The character of odor should always be recorded for future consideration.

B To eliminate psychological influences, the samples should be coded and intermixed so as not to suggest to the observer what odor concentration is being observed.

1 Bottles should be colored or covered with odor-free material or the observer blindfolded to eliminate auto suggestion

since many samples may possess color or turbidity.

2 Test should be conducted in a room free of outside odors. The observer should be cautioned to refrain from smoking or eating for an appreciable time before taking test. Odors should be washed from the hands prior to taking test.

3 The test should not be prolonged to a point where the sense of smell becomes fatigued.

IV PROCEDURE

A To obtain the approximate range of odor value take 50 ml, 14 ml and 5 ml of sample and make each sample up to 200 ml with odor-free water. Compare the odor of these three with 200 ml of odor-free water.

1 Cold odor: Bring dilutions to temperature of 24 - 25°C.

2 Shake each flask uniformly before smelling for odor. Observer should characterize type of odor.

3 Note which flasks contain odor and which do not. According to results obtained, prepare intermediate dilutions, in each case using sufficient odor-free water to make a total volume of 200 ml.

4 Include a flask with 200 ml of odor-free water with each series, as a blank for comparison.

B Arrange flasks so that their identity is unknown and bring to desired temperature.

1 Observe for odor and make chart with a "plus" or "zero" for each dilution.

2 The results are reported in "threshold odor numbers". The threshold odor number is calculated from the amount of sample in the most diluted portion which gives perceptible odor. The volume of the dilution (200 ml) divided by the volume of the sample in the dilution equals the threshold odor number. For example, if 5 ml diluted to 200 ml is the most dilute portion giving perceptible odor:

$$\frac{200}{5} = 40$$, the threshold odor is numbered 40.

C The threshold odor number shall not be confused with the "threshold odor concentration". The threshold odor concentration is the smallest amount of odor-producing material in mg/l required to give perceptible odor. If the threshold odor concentration is known, that value multiplied by the threshold odor number will give the concentration of the odor-producing material in the sample.

This outline was prepared by E. L. Robinson, Research Aquatic Biologist, Fish Toxicology Laboratory, 3411 Church Street, Newtown, OH 45244.

ROBERT A. TAFT SANITARY ENGINEERING CENTER
AQUATIC BIOLOGY

ALGAL THRESHOLD ODOR EXPERIMENT

Amount of Culture _____ ml Exp. No. _____

Age of Culture _____ days Temp. Tested at _____ °C

No. Cells per ml _____ Culture Medium _____

Mixed, Unialgal, Pure Date _____

Recorder _____

Observer No.			1	2	3	4	5	6	7
Observer									
Flask No.	Culture No.	Dilution No.	R	R	R	R	R	R	R
Threshold Odor No.									
Description of Odor									

\+ = Odor Detected O = No Odor Detected

Remarks

Estimated Composite* T.O. No. _____

*Geometric average of T.O. No. of individual observers E.L.R. 1956

COLLECTION AND INTERPRETATION OF BIOLOGICAL LAKE DATA

I INTRODUCTION

A The innumerable interrelationships of an organism with its environment are most complicated within the environs of a lake. The impact of these interrelationships upon a lake sampling program and upon the data collected must be recognized.

B Lake Sampling and Data Collection Entails:

1 A definition of the problem.

2 A determination of the types of samples necessary to point to a solution.

3 A delineation of sampling sites.

4 A judgment on the number of samples necessary.

5 A decision on the proper time and periodicity of sample collections.

6 Some knowledge or understanding of the science of limnology.

C In any lake sampling program the primary interest is in the relationship of the aquatic organism with its environment, and to fully appreciate this, one must have as a prerequisite some knowledge of the science of limnology.

II LIMNOLOGY - The study of inland waters

A Definitions of common terms:

1 Epilimnion - the turbulent superficial layer of a lake lying above the thermocline which does not have a permanent thermal stratification.

2. Thermocline - the layer of water in a lake between the epilimnion and hypolimnion in which the temperature exhibits the greatest difference in a vertical direction.

3 Hypolimnion - the deep layer of a lake lying below the thermocline and removed from surface influence.

4 Littoral - shorward region of a body of water; waters edge to the lakeward limit of rooted aquatic vegetation.

5 Sublittoral - from lakeward limit of rooted aquatic vegetation to the level of the upper limit of the hypolimnion.

6 Profundal - all of the lake floor bounding the hypolimnion below the light controlled limit of plant growth.

7 Limnetic or Pelagic - region of free water in which green plants are present only as phytoplankton.

8 Benthic - the region of the shore and the bottom of waters.

9 Trophogenic layer - the superficial layer of a lake in which organic production from mineral substances takes place on the basis of light energy.

10 Tropholytic layer - the deep layer of the lake where organic dissimilation predominates because of light deficiency.

11 Oligotrophic - waters with a small supply of nutrients and, hence, a small organic production.

12 Eutrophic - waters with a good supply of nutrients and, hence, a rich organic production.

13 Dystrophic - bog-type lakes containing yellow-brown water and much humus.

14. Liebig's law of the minimum - the yield of a plant or animal is determined by the quantity of that particular necessary substance which is present in minimal amounts as determined by the demands of the organism.

15. Seiches - oscillations of the water level--standing waves.

16. Standing crop - the total amount of biological growth present in the water on a selected date.

III FACTORS WHICH INFLUENCE SAMPLING AND DATA COLLECTION

A Physical Features

1. Temperature

a Lakes are warmed in spring principally by the action of wind forcing the warmer water down into the cooler water against the forces of gravity.

b Thermal stratification

2. Turbidity

3 Color

4. Water movement

5 Light penetration

a. A factor of turbidity, color, biological activity, and time of day

(1) Effective length of daylight dimenishes with the depth of the lake.

6 Wind velocity

7. Bottom materials

8. Size, shape, and slope of lake basin

B. Chemical Factors

1 Alkalinity, pH, and dissolved minerals excluding nitrogen and phosphorus

2 Dissolved oxygen

a From photosynthesis in sunlight

b From contact of lake surface with the air

c Fluctuates seasonally because of temperature and biological activity, and diurnally because of biological activity.

3 Nutrients for biological growth - especially nitrogen and phosphorus

a A given body of water will produce a given quantity of aquatic life. Biological production is determined primarily by the nutrients in solution in the water, and an increase in basic fertility will increase biological activity.

b Basic suppliers of nutrients include tributary streams, precipitation from the atmosphere, and interchange with lake bottom sediments.

C Biological Communities

1 The littoral community composed of rooted vegetation, attached algae, fish, and a host of invertebrates

2 The limnetic community composed of fish and plankton

3 The benthic community composed of midge larvae, sludgeworms, fingernail clams, and other bottom dwelling organisms

IV THEORY AND PRACTICE OF LAKE SAMPLING

A Purpose of Program

1 To point toward a logical and satisfactory solution to the specific problem.

2 To correlate the physical, chemical, and biological phenomena.

3 To reach an understanding of the interrelationships of the biota with the environment.

B Types of samples

1 Chemical

a Grab

b Composite

c Other

2. Biological

a Plankton

b. Benthic organisms

c. Fish

d Littoral vegetation

e. Bottom ooze

f Core samples of lake bottom

C Possible sample locations

1 Lake inlets and outlets

2 Random vertical samples in limnetic region away from littoral influence.

3 Samples collected at intervals on line transecting lake basin.

4. Individual "grab" or composited samples from similar regions and/or depths of lake.

5 Selected sites to define a specific problem.

D Number and periodicity of collections

1 The use of statistics is a valuable and necessary tool both in analyzing the data and in determining the number of samples necessary.

2 The question, "How many samples must I take?", is one of the more difficult ones facing the investigator. In reality, the availability of funds, personnel, and time are often the determining factors in a sampling program.

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This outline was prepared by K. M. Mackenthun, Biologist, formerly with Technical Advisory and Investigations Activities, FWPCA, SEC.

DETERMINATION OF PLANKTON PRODUCTIVITY

I INTRODUCTION

Primary production is the synthesis of organic matter from inorganic raw materials. The energy required for this process may come from light (photosynthesis), or from chemical sources (chemosynthesis). The primary synthesis of organic matter in lakes and streams is carried on by planktonic and benthic algae and bacteria, and aquatic macrophytes.

II PHOTOSYNTHESIS

The photosynthetic process involves the uptake of CO_2 and the release of O_2. The reactions are enzyme catalyzed and are affected by the following factors:

A Temperature

B Light Intensity

C Light Quality

D pH

E Nutrients

F Trace Elements

III MEASURING PRODUCTIVITY

Methods employed to measure plankton productivity are:

A Standing Crop

B Oxygen

C pH

D Carbon-14

IV STANDING CROP METHOD

The productivity of a body of water is indicated, in a general way, by the density of the plankton population. The standing crop of plankton is commonly measured by determining one or more of the following:

A Dry and Ash-free Weight of Seston

B Cell or Unit Counts

C Cell Volume

D Chlorophyll

E Particulate and Dissolved Carbohydrate

F Particulate and Dissolved Organic Carbon

Increases in the standing crop over a period of time may be used to determine productivity. However, this method provides only a rough approximation of the rate of primary production.

V OXYGEN METHOD

The use of dissolved oxygen to determine short-term rates of primary production was introduced by Gaardner and Gran (1927). Estimates of the amount of carbon fixed are based on the premise that one molecule of oxygen is given off for each atom of carbon assimilated.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

A "Light" and "dark" bottles are filled with sample and resuspended at various depths for 4 - 24 hours.

B The concentration of dissolved oxygen is determined (using the Winkler Method) at

the beginning and end of the incubation period. The values obtained are as follows:

1 Final "light" bottle O_2 - initial O_2 = net photosynthesis

2 Initial O_2 - Final "dark" bottle O_2 = respiration

3 Net photosynthesis + respiration = gross photosynthesis

This method has some serious disadvantages:

A The bottles provide an artificial substrate for the proliferation of bacteria which use up large amounts of O_2, resulting in erroneously high respiration and low net photosynthesis values.

B The lower limit of sensitivity of the Winkler Method is 0.02 mg O_2/liter. This is a serious handicap when working in oligotropic lakes and the open sea.

VI CARBON-14 METHOD

The use of carbon-14 for the measurement of the rate of carbon assimilation by phytoplankton was pioneered by E. Steemann Nielsen (1952). The method is simple and very sensitive.

A Carbon-14 labelled sodium bicarbonate (4 - 10μc/liter) is added to "light" and "dark" bottles, which are resuspended in the water for 4 - 24 hours.

B An aliquot of the sample is passed through a membrane filter (1.2 μ pore diameter), and the filters are treated with acid to remove any inorganic labelled carbon.

C The (beta) activity of the filter is determined with an end-window Geiger tube, or with gas flow or liquid scintillation techniques.

D The carbon fixed is determined as follows:

$$\text{carbon fixed} = \frac{\text{activity on filter}}{\text{total activity added}} \times \text{available } HCO_3^- \times \text{correction for isotope discrimination}$$

There are several important disadvantages in this method.

A Some of the labelled photosynthesis products will be broken down immediately by respiration, and the liberated carbon-14 reused in photosynthesis. Therefore, it is generally agreed that the method measures only net photosynthesis.

B It has been found that the algae rapidly excrete up to 50% of the photosynthate in the form of organic acids, carbohydrates, and amino acids. Since these labelled materials are not retained by the filter, they escape detection.

VII pH METHOD

The uptake of CO_2 by the algae during photosyntheses results in an increase in the pH of the surrounding medium. Periodic pH measurements are made of the body of water being studied, and the carbon uptake is determined using published nomographs.

Verduin (1952) used this method in a study of the productivity of Lake Erie. However, the method has not gained wide acceptance because it can be used only in waters with low alkalinity.

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This outline was prepared by C. I. Weber, Chief, Biological Methods Branch, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

AERIAL RECONNAISSANCE IN POLLUTION SURVEILLANCE

I INTRODUCTION

A Definition

The word "reconnaissance" is derived from the word "reconnoiter" which means to conduct a preliminary examination or survey. Its earlier applications to engineering and military requirements has been expanded to include photomapping and interpretations of natural resources. Aerial reconnaissance can be defined as "airborne examination or survey procedures performed by heavier-than-air craft, lighter-than-air craft, or earth orbiting satellites."

B Types of Aerial Reconnaissance

1 Visual

Examination of the flight path by a human observer with no provision for permanent recording for later study. This form of aerial observation has limited use but in many cases can complement the other forms.

2 Image forming sensor

Image recording of the covered flight path where maximum advantage can be made of image interpretation techniques. Image forming sensors includes such instruments as cameras, infrared scanners, and radar.

3 Nonimage forming sensors

Nonimage forming sensors include such devices as oscilloscopes, strip charts, and dial indicators which directly indicate parameter differentials as received by the sensing elements from the target scan.

4 Combination image forming and nonimage sensors

It is advantageous at times to combine both of these techniques in order to rapidly interpret flight path imagery. This advantage is shown in Figure 1 where a heated effluent discharge is traced to a canal by infrared imagery and indicated by the "lighter" plume which parallels the shoreline. The oscilloscope phototracing indicates the temperature differential of the discharge point as compared to the offshore bay area.

C Detected images can be identified by one or more of the following criteria.

1 Size of image

2 Shape of image

3 Tonal qualities of image

4 Image profile shadowing

5 Location of image

6 Texture patterns of grouped images

7 Spatial relationship of image to surrounding bodies

D Selection of Remote Sensing Method

1 A knowledge of the electromagnetic spectrum (Figure 2) is important in order to select the most advantageous method of remote sensing. It will be noted that there are transmissibility and sensitivity limits as well as existing film limits with respect to equipment wave length patterns.

2 Remote sensing methods can be categorized into two areas.

a Source active: Utilization of an instrument which is capable of emitting a source of energy which is transmitted to the target area and emanations are received whose characteristics are dependent upon the nature of the specific target.

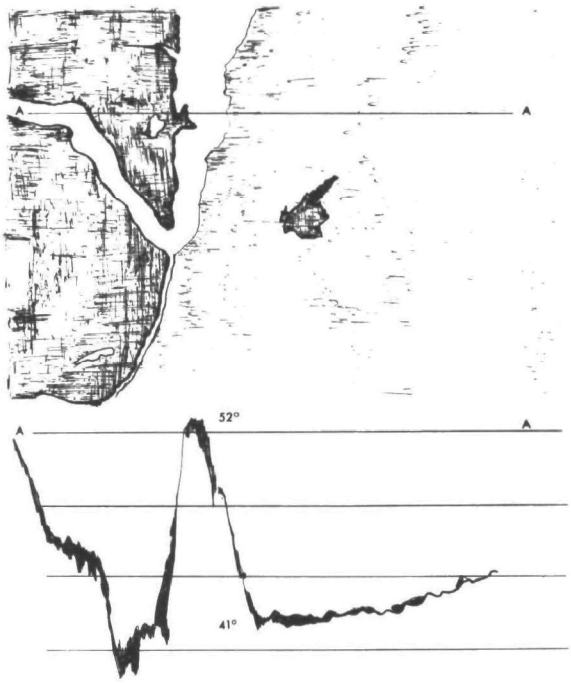

FIGURE 1

An example of such a device is the Radar set (Figure 3) or special Infrared Emitters both of which are capable of being utilized in total darkness.

b Source passive: Utilization of instrumentation which is only capable of receiving target area emanations. Such remote sensing instruments, therefore, do not transmit artificial energy sources but depend upon the sun as a source of energy which is selectively received and individually reflected by each object in the target area. Aerial photography and most of the remote sensing is by this method and the included regions of the electromagnetic spectrum are the upper portions of the UV band to the near infrared region.

3 Selection of desired wavelength is dependent upon the optimum emission capability of the instrument which has the shortest wavelength possible to sharply differentiate small objects while being long enough to preclude excessive energy "scattering" and yet not long enough so that photographic recording is impossible.

II TECHNIQUES

A Radar(RAdio Detection And Ranging)

Radar is particularly useful for discerning certain types of vegetation which may not appear in the best visual quality in Panchromatic Color Film (sensitive to the entire visible spectrum) and also has all-weather and around-the-clock capabilities. Radar in the longer wavelength ranges is capable of penetrating dense vegetative cover and this advantage has been found to be useful in detecting drainage networks and geological features.

B Conventional Photography

Conventional photography covers the entire range of the visible spectrum and employs both black-and-white film and color film. Some of the special films are capable of photographing a portion of the infrared band (the "near" infrared region) and this capability is useful in special situations.

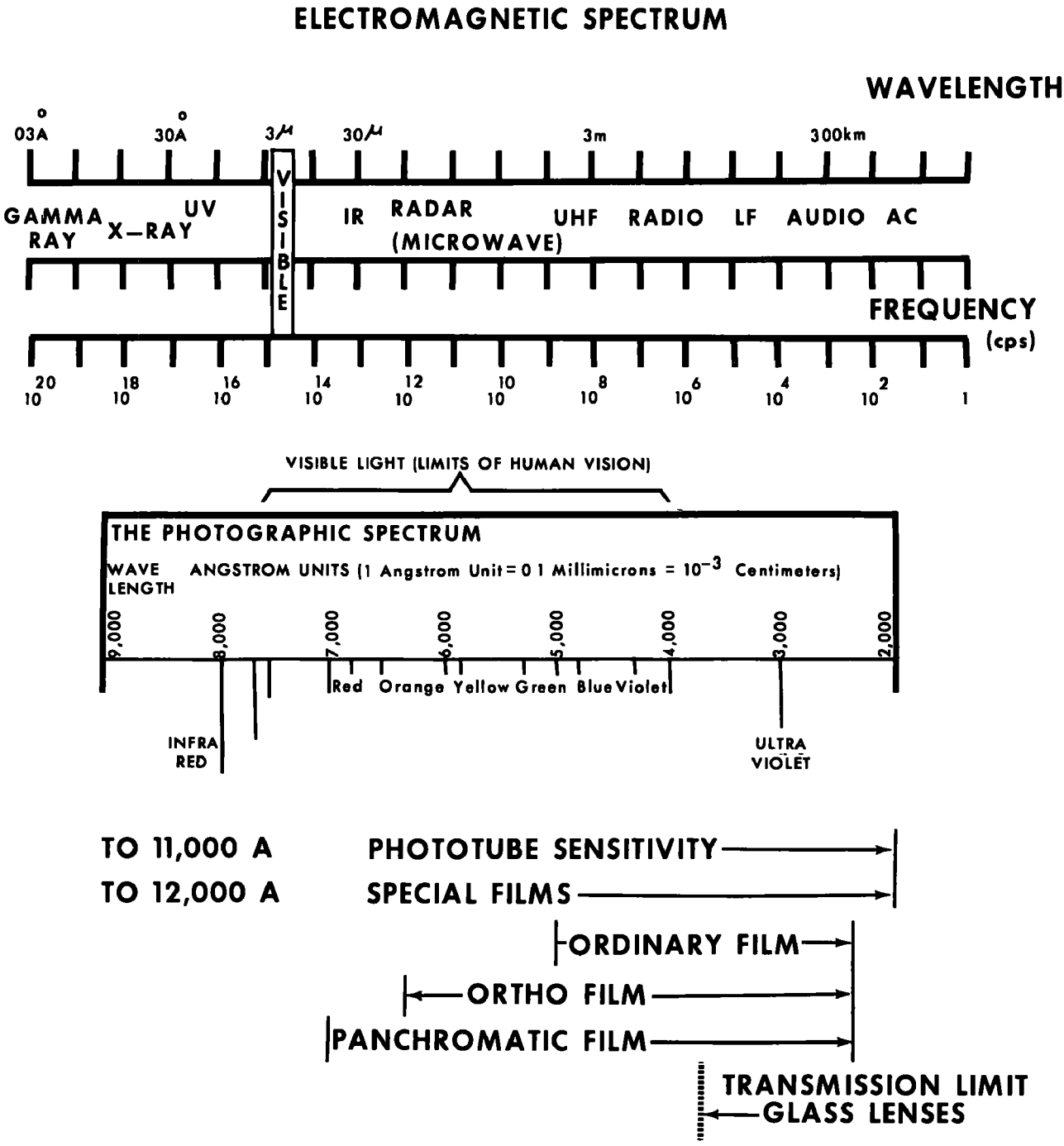
ELECTROMAGNETIC SPECTRUM
WAVELENGTH
03A
30A
3μ
30μ
3m
300km
GAMMA RAY
X-RAY
UV
VISIBLE
IR
RADAR
(MICROWAVE)
UHF
RADIO
LF
AUDIO
AC
FREQUENCY
(cps)
20 18 16 14 12 10 8 6 4 2 1
10 10 10 10 10 10 10 10 10 10
VISIBLE LIGHT (LIMITS OF HUMAN VISION)
THE PHOTOGRAPHIC SPECTRUM
WAVE LENGTH
ANGSTROM UNITS (1 Angstrom Unit = 0 1 Millimicrons = 10⁻³ Centimeters)
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
Red Orange Yellow Green Blue Violet
INFRA RED
ULTRA VIOLET
TO 11,000 A
PHOTOTUBE SENSITIVITY
TO 12,000 A
SPECIAL FILMS
ORDINARY FILM
ORTHO FILM
PANCHROMATIC FILM
TRANSMISSION LIMIT
GLASS LENSES

FIGURE 2

FIGURE 3

1 Direct photomapping and panoramic scan

Figure 4 illustrated the manner in which direct photomapping differs from the panoramic scanning technique and it can be seen that continual attached mapping scans can be made of all or portions of the flight path to be analyzed.

2 Multiband photography

Multiband photography is, as its name implies, the synchronized photography of up to nine separate photographs of the same target area each of which is photographed in a different wavelength. A distinct advantage of this method is that, during photo analysis, distinct anomalies may be evident between objects appearing in the different bands and further investigation may be warranted. An example of this is shown in Figure 5 where it was observed that an anomaly in vegetative growth was apparent in the Infrared wavelength while not being apparent in the other visual spectral ranges. Further investigations proved that gravel washings in slug intervals had affected normal growth of the streamside vegetation.

3 Infrared photography

Infrared photography has been found useful in depicting certain hydrological features. Within the photographic ranges it is not a heat-measuring tool and therefore cannot detect thermal anomalies within bodies of water but instead sharply delineates shoreline and tributary features and has haze penetrating qualities. The blues appear much darker by infrared photography and the reds, greens, and yellows will show up much lighter when compared to a panchromatic color photograph.

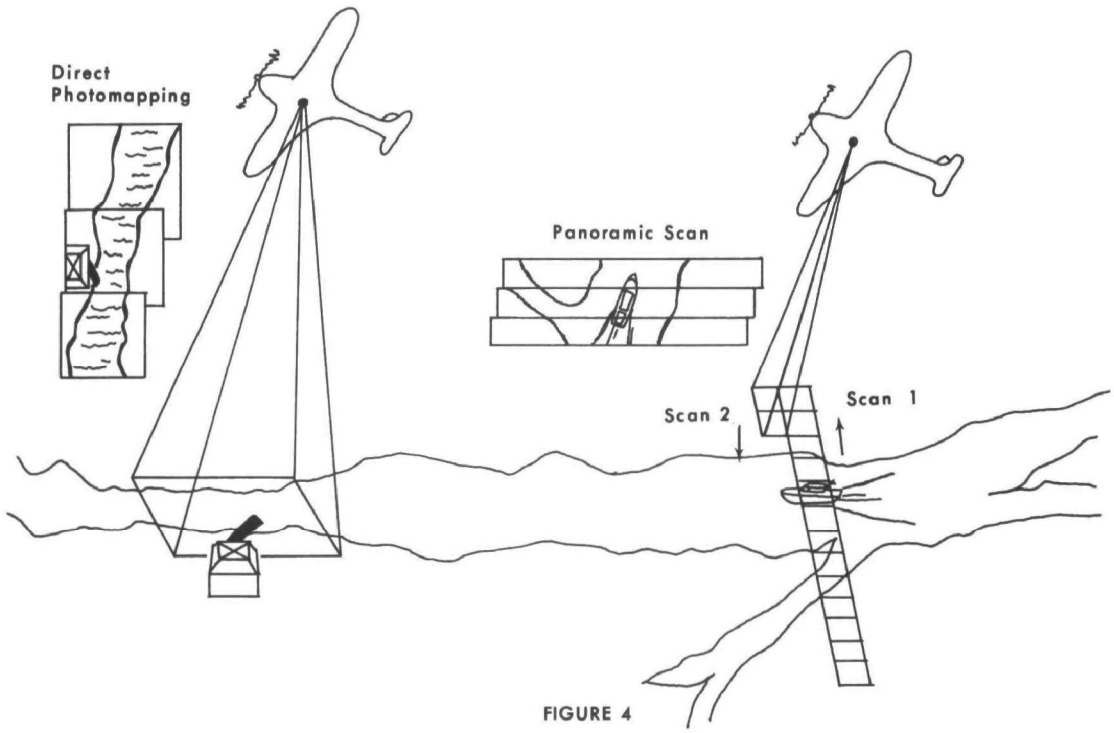

FIGURE 4

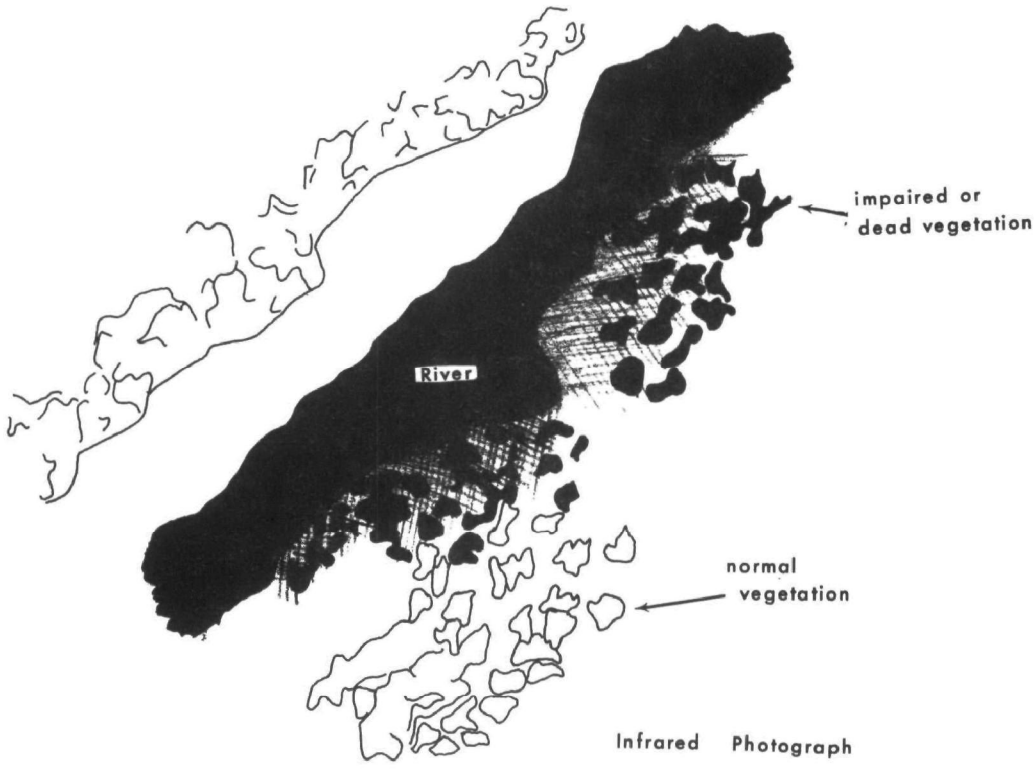

FIGURE 5

4 Gamma Ray Spectrometer

This device functions in the very short wavelengths and has been found to have excellent detecting capabilities for radioactivity. Such a device would serve as an excellent means of searching for radioactive waste spills which have occurred either accidentally or intentionally.

C Infrared Radiometry

Infrared imagery is different than infrared photography since the tone of the imagery is directly related to the infrared radiation emitted from the target area. This type of scanner converts the normally unphotographic IR band to a thermal imagery. An optical-mechanical device is necessary since the normal thermal energy emitted by the camera itself will influence radiations received from the target area and this elimination of detector internal radiation is accomplished by miniturization and application of extremely low temperatures. One example of the usefulness of infrared imagery is shown in Figure 6 where the dark areas along the shoreline were found to be caused by cold water infiltration by a previously unknown source. Such a finding could be indicative, for example, of a new untapped freshwater source or a polluting influence from a remote source. This cold water infiltration was not evident by normal visual spectrum photography and the IR photograph would only indicate a more distinct shoreline.

D Automatic Analysis

In the analysis of large numbers of prints use can be made of photoelectric scanners wherein automatic recording of degree of brightness can be recorded on a tape printout. An example of this procedure is shown in Figure 7 where each symbol has a significance relating to "tone signature." The "D" zones are the darker areas while the other symbols relate to lighter zones. In the center the clear area is a river tributary. Future predictions are that automatic encoding can be channeled to computers wherein decisions can be made, for instance, in water management policies.

E Stereoscopic Analysis

The principles and methods developed during World War II for the stereoscopic analysis of aerial photographs has been a continuing and expanding science. Its principles are based upon the fact that the human eyes are normally spaced about 65 millimeters apart and are capable of compositing, via the optic nerve, the two separate and distinct images viewed by each orbit. Aerial stereoscopic analysis depends upon the placing of two photographs precise distances apart with precise overlapping as required by normal human visual acuity. With the aid of special stereoscopic viewing lenses the viewer is able, in most cases, to composite both of the photographs in the same manner as with normal vision and thus a sense of depth can be imparted materially enhancing the analyzing of the terrain.

III APPLICATIONS

Aerial reconnaissance in pollution surveillance can be accomplished in a variety of ways and the method chosen usually is dictated by costs involved and what is desired with regard to immediate or potential information.

A Visual Observations

The simplest and least expensive method is to visually observe the study area and this can be useful in determining sampling sites, locating previously undetermined tributaries, pinpointing visual gross pollutant effluents, etc. To augment these observations it is usually better to have a permanent recording of the survey area in the form of photographic or imagery recordings.

B Photographic Techniques

Photographic evidence, either color, black-and-white, or special film, can be recorded in various attitudes or positions of the aircraft in order to make best use of interpretation techniques such as stereoscopic analysis.

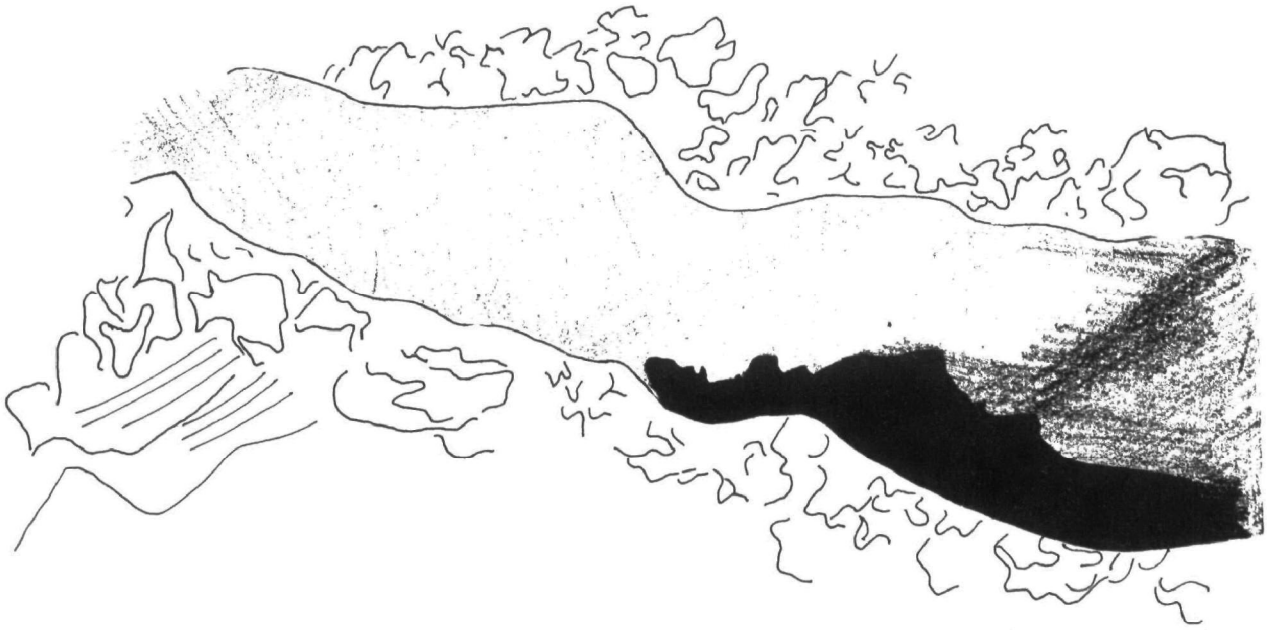

FIGURE 6

FIGURE 7

1 Current patterns and flow velocity can be ascertained by a method developed by the U.S. Coast and Geodetic Survey whereby powdered aluminum is surface distributed to a wide area and subsequent photographic patterns can be analyzed. Flowing bodies can either appear as "depressions" or "elevations" depending upon direction of flow with relation to the photographing aircraft and from these "parallax" anomalies the velocity can be determined.

2 Photographic IR has found applications in the areas of detecting sharp delineations of shorelines and tributaries especially when haze is prevalent.

3 The work of Strandberg[1] concludes that "In aerial photographs, oxygen-deficient water frequently appears black, or at least darker in tone and this appearance may be caused by the incomplete reduction of wastes by anaerobic bacteria." Thus his black-and-white photographs of waterbodies with low values of dissolved oxygen (DO) following a source of waste discharge are characterized by a plume discharge followed downstream by dark water tones.

Further values ascribed to photography in this manual include color photographs dealing with fish kills where the extent of damage can be better assessed and the use of "false" color infrared photography where algal masses can be differentiated and, again, the low DO concentration is manifested by a color anomaly.

C Imagery Techniques

1 Utilization of the "mid" and "far" infrared wavelengths in the form of "imagery" has been found useful in analyzing for thermal pollution and its concomitant circulation and diffusion patterns for which "thermographs" can be described for large water bodies.[2] It is also possible to establish naturally occurring patterns of seasonal and diurnal thermal ranges for surface waters.

2 It has been ascertained, in principle, that it is possible to identify pollutants by study of spectral emissivity characteristics for particular wavelengths and this can be of value, for instance, when one is searching for a particular pollutant and the emissivity characteristics for the waterbody can be compared with known patterns for the specific pollutant.[2]

3 Utilization of radar and orbiting satellite platforms can produce imagery of great usefulness. The radar technique can precisely delineate shoreline characteristics without vegetative interferences and thus can thoroughly image drainage basins. Satellite imagery and photography can obtain the same information but on a much broader scale and with great rapidity. Recent lines of thought have postulated that this orbiting scanner can be fitted with computing devices to render decisions upon analysis of its constant data collection.[3] Such decisions can be of immeasurable aid to the water conservationist who may require long range predictions based upon analysis of whole river basins.

IV SUMMARY

Development of aerial reconnaissance in pollution surveillance has proceeded in the areas of technique and interpretation. With regard to the techniques available most of the earlier problems have been overcome to a degree where available material is amenable to analysis. Such early problems as pitch and yaw of the aircraft, cloud cover, imagery instrumentation, etc., has been overcome to a large degree by available engineering skills and the need for improvement due to military needs. In the area of interpretation, however, much has yet to be accomplished to develop this tool as an extension of laboratory analysis which it will augment rather than replace.

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This outline was prepared by R. Russomanno, Microbiologist, National Training Center, FWPCA, Cincinnati, OH 45226.

LABORATORY EXERCISES

Proportional Counting of Plankton	11
Calibration of Plankton Counting Equipment	12
Fundamentals of Quantitative Counting	13
Class Problem in Plankton Analysis	14

LABORATORY: PROPORTIONAL COUNTING OF PLANKTON

I OBJECTIVE

To learn and practice the techniques of proportional counting of mixed plankton samples.

II MATERIALS

A Several plankton samples, each containing a number of plankton forms.

B Class slides, cover slips, and dropping pipets.

III PROCEDURES

A Make an ordinary wet mount of the sample provided.

B Scan the slide. Identify and list all types of plankton present.

C Proportional Counting (use clump count)

1 Field count

a Count and tally all individuals of each type present in a field. The best way to do this is to list the most common types separately and record the counts and then enumerate the other forms.

b Move the slide at random and repeat the process. Do this for 5 or 10 fields.

c Tally the results and compute the percent of each type.

2 Five hundred count

a Moving the slide at random count and tally all the types of plankton as before until a total of 500 cells or clumps have been counted.

b Tally the results and compute the percentage of each type as before.

IV RESULTS

A Record your results for both methods on the board.

B Discuss the two methods and the use of the proportional count results.

This outline was prepared by M.E. Bender, Biologist, formerly with FWPCA Training Activities, SEC.

LABORATORY: CALIBRATION OF PLANKTON COUNTING EQUIPMENT

I OBJECTIVES

A To Become Familiar with Microscope Calibration Procedures

B To Calibrate the Particular Equipment Assigned to you

II MATERIALS

A Whipple, Plankton Counting Reticule

B Compound Microscope as Assigned

C Stage Micrometer

III PROCEDURE

A Adjust the interpupillary distance to the position most comfortable for your eyes, and record the setting on the "Microscope Calibration Data" sheet.

B Install a Whipple plankton counting reticule in the right eyepiece.

C Obtain a stage micrometer and focus on the scale at 100X magnification.

D Record the exact dimensions of the entire field in the column marked "Whole" on the plate "Microscope Calibration Data."

E Do the same with the 200X and 400X magnifications.

F Return the stage micrometer to the supply table.

G Values for the "Large" and "Small" columns may now be calculated arithmetically. There are ten large squares across the whole field, and 5 small squares across the large square which is subdivided, in the center of the field.

H Calculate the conversion factors to counts per ml according to the formulae in the lecture entitled "Calibration and Use of Plankton Counting Equipment."

This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

MICROSCOPE CALIBRATION DATA

Microscope No. _____

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with Objective Serial No. _____ and Ocular Serial No _____					(2 S-R Strips)
200X, obtained with Objective Serial No. _____ and Ocular Serial No. _____					(2 S-R Strips)
400X, obtained with Objective Serial No. _____ and Ocular Serial No. _____					(Nannoplankton) (cell-20 fields)

*1mm = 1000 microns

BI. AQ. pl 8 10. 60.

MICROSCOPE CALIBRATION DATA

Microscope No _____

Approximate Magnification	Tube Length, or Interpupillary Setting	Linear dimensions of Whipple squares in millimeters*			Factor for Conversion to count/ml
		Whole	Large	Small	
100X, obtained with Objective Serial No. _____ and Ocular Serial No _____					(2 S-R Strips)
200X, obtained with Objective Serial No. _____ and Ocular Serial No. _____					(2 S-R Strips)
400X, obtained with Objective Serial No. _____ and Ocular Serial No. _____					(Nannoplankton) (cell-20 fields)

*1mm = 1000 microns

BI. AQ. pl 8 10. 60.

LABORATORY: FUNDAMENTALS OF QUANTITATIVE COUNTING

I OBJECTIVE

To learn and practice the basic techniques of quantitative plankton counting

II MATERIALS

A Plankton Samples Containing a Variety of Plankton Forms

B S-R Cells and Coverglasses, Large Bore 1 ml Pipettes, Whipple Discs, Plankton Record Form

III PROCEDURE

A Fill the S-R cell with sample number 1 as follows:

Place the coverglass diagonally across the S-R cell This leaves the other two corners uncovered; one for putting in the sample fluid, the other to allow air to be driven out as it is replaced by the incoming aliquot. Shake the sample to disperse the plankton. Before settling occurs in the sample draw about 1-1/4 ml of the fluid into the pipette and quickly fill the S-R cell by delivering the aliquot into one of the open corners of the chamber.

B Using 100x focus on the sample. After focus has been obtained switch to 200x. Scan the slide and list the plankton forms present.

C Starting from one end of the S-R cell and proceding to the opposite (this is called a strip count, begin counting (clump counts) the plankton forms. The length of the cell may be traversed in several ways.

1 Count all the forms in the Whipple square or in a portion of the square, record the count and move the slide so that the square covers the adjoining area.

2 Move the slide very slowly counting and recording the various forms as they pass the leading edge of the Whipple disc.

IV RESULTS

A Using the correction factor obtained in the previous laboratory compute the number of plankton organisms per ml.

B Record the results on the board.

C Discussion of Results

D Refill the slide with a fresh aliquot and recount the sample. Compare results with the first count.

E Count the other samples of mixed plankton as assigned, following the same procedure.

This outline was prepared by M.E. Bender, Former Biologist, FWPCA, Water Pollution Training Activities, SEC.

SHEET NO. _____

PLANKTON COUNT RECORD

Body of Water _____ Date Collected _____ Date Analyzed _____

Station _____ Depth _____ Collector _____ Analyst _____

	Notes, Tallys	Total	Vol, Area, or Type of Count	Magnification	Multifactor	Count per ml	Count per liter	Group Totals
TOTAL COUNT								
Organisms, Differential Count ↓								

FIELD CONDITIONS

Time of Day _____

Air Temp _____ Water Temp _____

Weather today _____

Previous Weather _____

Turbidity Method _____ T reading _____

Method of Collection _____

Total Vol Collected _____

Preservative _____

General Conditions _____

Filamentous Algae _____

Other Plants _____

Surface Scum _____

Dead Fish _____

Odor of Water _____

Other Physical or Chemical Data _____

_____

LABORATORY ANALYSIS

Method of Preparation _____

Departure from Routine _____

_____

Significance of Results _____

_____

Treatment Recommended _____

_____

PLANT OR OTHER DATA, FOR EX

Treatment to Date.

Act C _____ cl _____

Other Chemicals _____

Result of Treatment

Taste and Odor

Raw _____ Finished _____

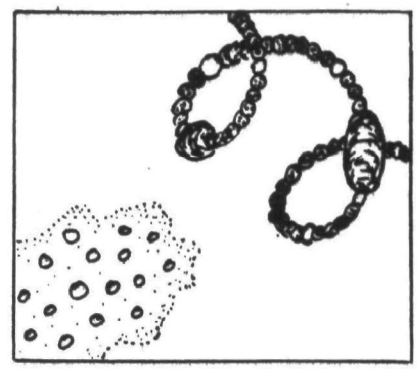
Filter Runs _____

Other _____

SUGGESTED BASIC FORM FOR PLANKTON RECORDS

HWJ
4/68

DI MIC enu pl 2 4 68



CHAPTER IV

INTERPRETATION AND SIGNIFICANCE OF PLANKTON

Algae and Actinomycetes in Water Supplies	1
Algae as Indicators of Pollution	2
Public Health Significance of Toxic Algae	3
Odor Production by Algae and Other Organisms	4
Organic Enrichment and Dissolved Oxygen Relationships in Water	5
Plankton in Oligotrophic Lakes	6
The Effects of Pollution on Lakes	7

ALGAE AND ACTINOMYCETES IN WATER SUPPLIES

I Water treatment always should include detection and control of microorganisms.

A Two types of microorganisms are involved:

1 Pathogenic types include such forms as the typhoid bacteria, the dysentery ameba, and the infectious hepatitis virus.

2 Interference types include taste and odor organisms, filter-clogging organisms, pipe-infesting organisms, and others.

B Water treatment practices are closely associated with these organisms.

1 For pathogens, practices include coliform tests, use of chlorine, and guarding the water supply against fecal pollution.

2 For interference organisms, practices include plankton enumeration, use of copper sulfate and the covering of reserviors.

3 Many of the other treatment practices have significant effects on the organisms.

C This discussion will be limited to the interference organisms.

II EXAMPLES OF PROBLEMS CAUSED BY INTERFERENCE ORGANISMS

A At Chicago, the alga Dinobryon reappears almost every year, generally in June and July in numbers sufficient to impart a prominent fishy odor to the water. In 1951, it required an estimated $70,500 worth of activated carbon to control the odor of this organism for a period of two months.

B At Indianapolis, copepods were present in parts of the distribution system in numbers sufficient to be visible in the drinking water. The eggs of the copepods were found to pass through the filters and to hatch in the distribution system.

C At Oklahoma City, prominent earthy odors have appeared frequently. The organisms blamed for this trouble are the mold-like actinomycetes.

D At Peoria, white wigglers up to 3/8" long were reported in the tap water, during early March, 1956. These chironomid larvae had hatched in the city's open reservoir, requiring that the reservoir be drained, cleaned and treated with a larvicide.

E At Chicago, diatoms are a very important cause of short filter runs. The one diatom Tabellaria is considered to be more responsible than any other organism for this trouble.

F In Ontario, the alga Cladophora often grows in large numbers attached to rocks on the shoreline of lakes. When the alga is broken loose it collects near the shoreline and gives rise to very offensive odors.

G In a water supply impoundment in Utah the plankton algae frequently cause the pH of the water to increase to 8.3 or higher, requiring that the water be treated with acid to obtain the desired pH of 8 or lower.

H In Texas a water supply from underground sources was stored in a large open settling basin. Oscillatoria and unicellular green algae developed in large numbers in the stored water, turning it green and producing a strong odor.

I Los Angeles has more than 25 open reservoirs of various sizes and ranging in elevation from almost sea level to over

7,000 feet. Many tons of copper sulfate are used every year in these reservoirs for rigid control of plankton, chiefly diatoms and occassionally blue-green algae. This treatment is carried out to improve the water quality including the reduction of tastes and odors.

III TYPES OF PROBLEMS CAUSED BY INTERFERENCE ORGANISMS

A Tastes and Odors

1 May be caused by algae, actinomycetes, crustacea, and anaerobic bacteria.

2 Common algal odors imparted to water are ones described as fish, earthy, musty, grassy, cucumber, geranium, nasturtium, and septic.

3 Common actinomycete odor is earthy.

4 Tastes produced in water by algae include sweet and bitter.

5 Other causative agents of tastes and odors may be industrial wastes, sludge, and compounds dissolved from soil and rock, and chemicals used in treatment.

B Filter Clogging

1 Both rapid and slow sand filters are affected.

2 Diatoms are the organisms most frequently involved but blue-green algae, filamentous green algae and other organisms as well as silt may cause it.

C Other Problems in the Treatment Plant

1 Algae may cause variation in the pH, hardness, color, and organic content of the water.

2 Amount of plankton organisms often influences the rate and effectiveness of coagulation.

3 Chlorine dosage may depend upon amount of plankton organisms present.

4 Growths of algae may reduce the flow through influent channels and screens.

5 Organisms may be responsible for increasing the quantity of sludge to be disposed of in sedimentation basins.

6 Microcrustacea "spot" paper in paper mill rolls.

D Infestation of Distribution Systems

1 Attached organisms reduce the rate of flow in the pipes.

2 Iron and sulfur bacteria may initiate or stimulate corrosion of pipes.

3 Organisms may appear as visible bodies in tap water.

4 Tastes and odors may result from presence of organisms.

5 Chlorine residual is difficult to maintain when organic matter is present.

6 Organisms could theoretically harbor and protect against chlorine certain pathogenic bacteria.

E Profuse Growths of Organisms in Raw Water Supplies

1 A limited and balanced growth of various organisms is generally an asset.

2 Extensive surface mats, blooms and marginal growths often cause troubles along the shoreline and eventually in the treatment plant.

3 Some fish kills may be caused by profuse growths of algae by reducing the DO during the night.

4 Certain massive growths of blue-green algae are deadly poisonous to animals.

IV ORGANISMS INVOLVED

A Animal forms include protozoa, rotifers, crustaceans, worms, bryozoans, fresh water

sponges, water mites and larval stages of various insects.

B Plant forms include algae, actinomycetes and other bacteria, molds and larger aquatic green plants.

V IMPORTANCE OF BIOLOGICAL PROBLEMS

A The increased use of surface water supplies increases the problems caused by organisms. Biological problems are less common with ground water supplies.

B Standards of water quality requested by domestic and industrial patrons are rising.

C Procedures for detection, control and prevention of problems caused by organisms are improving and are receiving more extensive use.

VI A number of methods may be used to control the interference organisms or their products:

A Addition to water or an algicide or pesticide such as copper sulfate, chlorine dioxide or copper-chlorine-ammonia.

B Mechanical cleaning of distribution lines, settling basins, sand filters, screens, and reservoir walls.

C Modification of coagulation, filtration, chemical treatment, or location of intake.

D Use of absorbent, such as activated carbon, for taste and odor substances.

E Modification of Reservoir to Reduce the Opportunities for Massive Growths of Algae

1 By covering treated water reservoir to exclude sunlight

2 By increasing the depth of the water in reservoirs

3 By eliminating shallow marginal areas

4 By reducing the amount of fertilizing nutrients entering the reservoir.

VII It is generally more satisfactory to anticipate and prevent problems due to these organisms than it is to cope with them later.

A Routine biological tests are essential to detect the initial development or presence of interference organisms.

1 Control measures can then be used before problem becomes acute.

2 These tests should be applied to the raw treatment plant water supply and distribution system.

B In the Reservoir or Other Raw Water Supply

1 Routine plankton counts should be made of water samples from selected locations. Plankton counter should be aware of the particular organisms known to be most troublesome.

2 During the warmer months routine surveys of the reservoir, lake or stream should be made to record any visible growths of algae and other organisms.

3 Odor tests of water from several locations should be made to obtain advance notice of potential trouble at the treatment plant.

C In the Treatment Plant

1 Records of plankton counts and threshold odor between each step in treatment gives data on effectiveness of each procedure.

2 Coagulation and filtration can be adjusted to remove up to 95% or more of organisms in water.

3 Microscopic analysis of samples of filter material for organisms can supply data useful in modifying sand filtration and treatment of finished water.

D In the Distribution System With Its Finished Water

1 Open reservoirs require constant attention especially during summer.

2 Parts of the system farthest from the treatment plant or adjacent to dead ends require most frequent sampling for organisms and tastes and odors.

VIII SUMMARY

A Interference organisms cause problems in distribution systems, treatment plants, raw water supplies.

B Organisms involved include algae, actinomycetes, other bacteria, and minute aquatic animals.

C Control is by special chemicals, mechanical cleaning, adjustment of chemical or mechanical treatment and by modification of reservoirs, intakes, etc., for the raw water supply.

D Facilities for detection of problems in their early stages are required for most efficient and satisfactory control.

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This outline was prepared by C.M. Palmer, formerly Aquatic Biologist, In Charge, Interference Organism Studies, Microbiology Activities, Research & Development, Cincinnati Water Research Laboratory, FWPCA.

ALGAE IMPORTANT IN WATER SUPPLIES

TASTE AND ODOR ALGAE

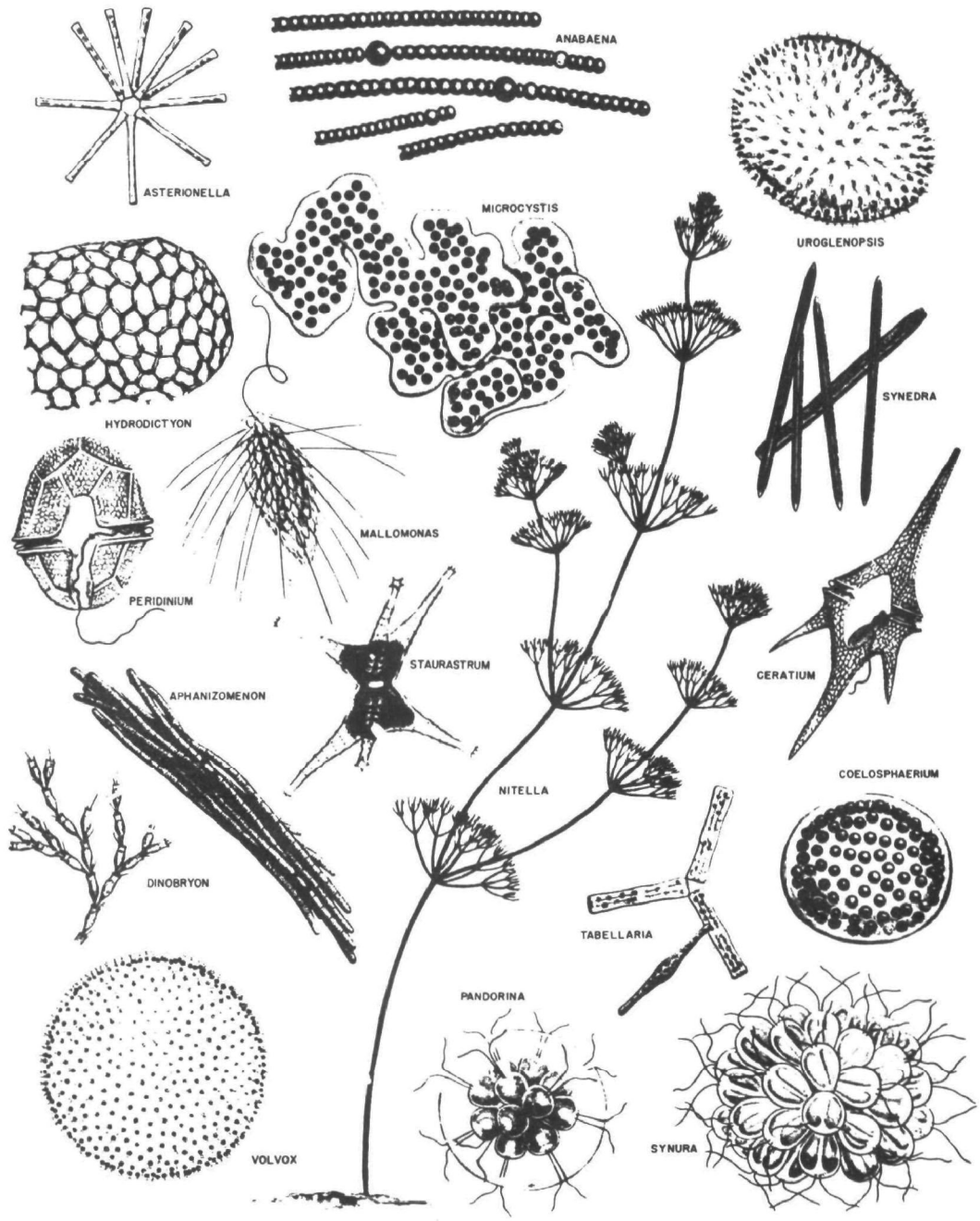

PLATE I

FILTER CLOGGING ALGAE

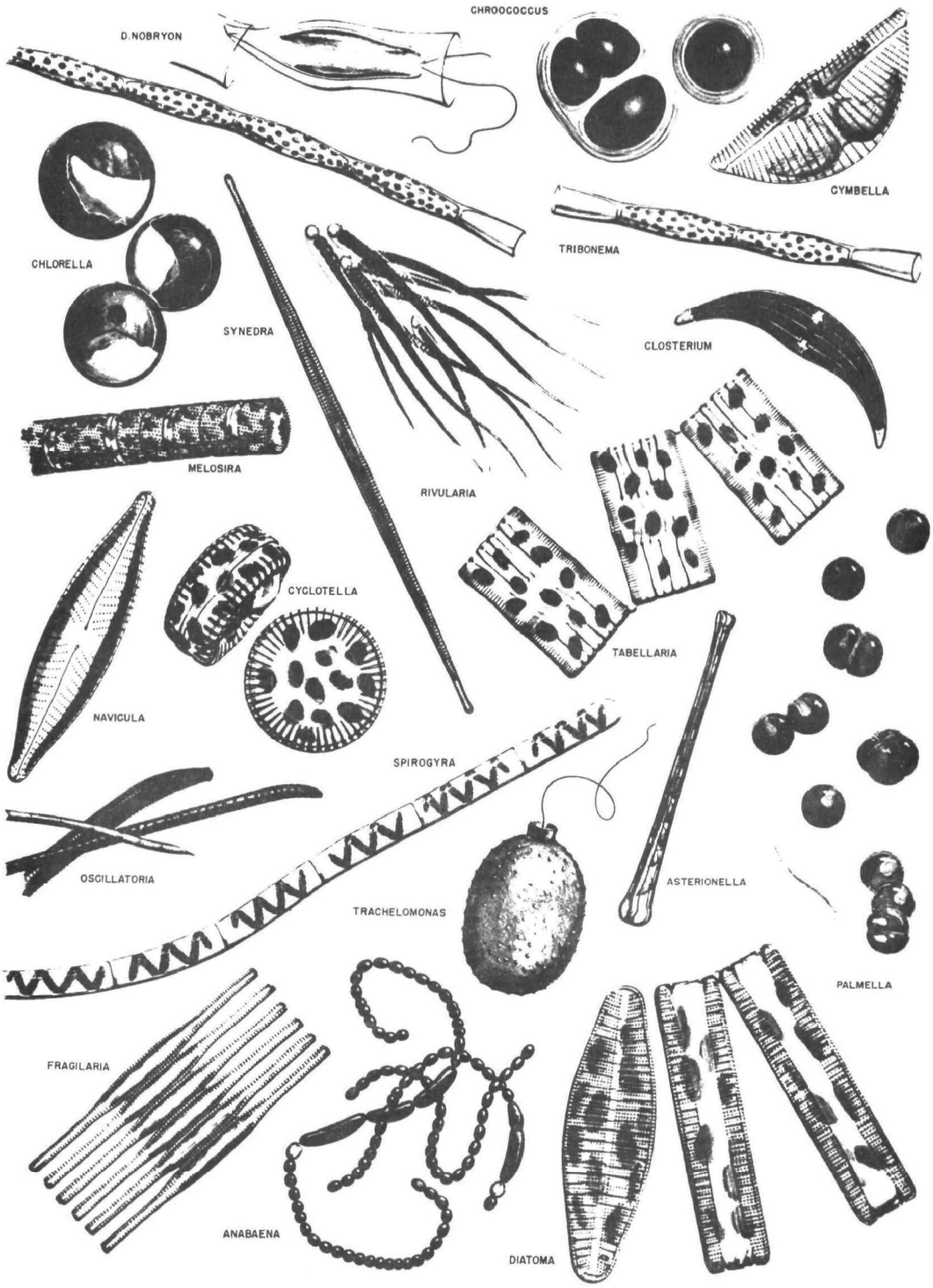

PLATE 2

POLLUTED WATER ALGAE

PLATE 3

CLEAN WATER ALGAE

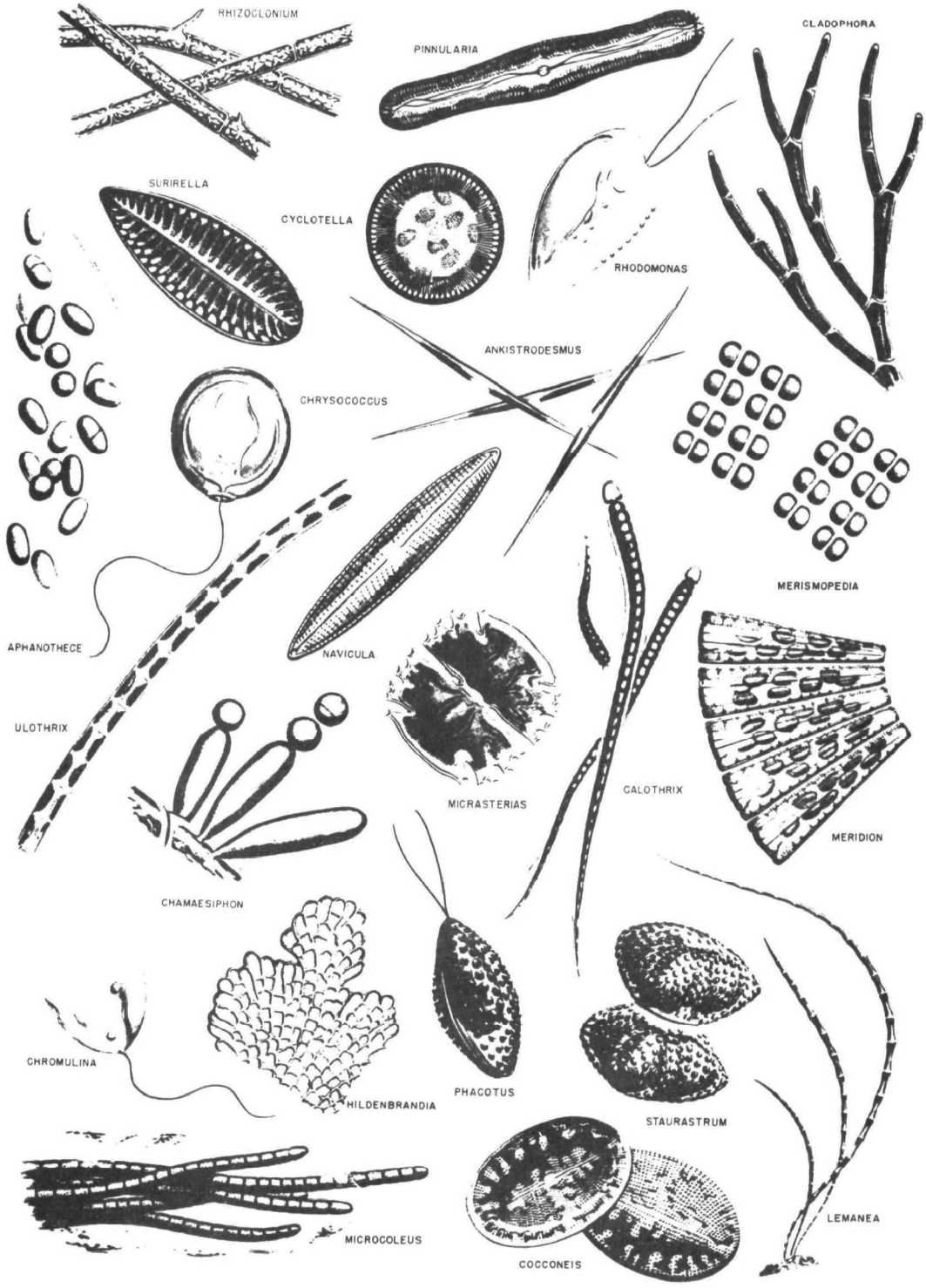

PLATE 4

SURFACE WATER ALGAE

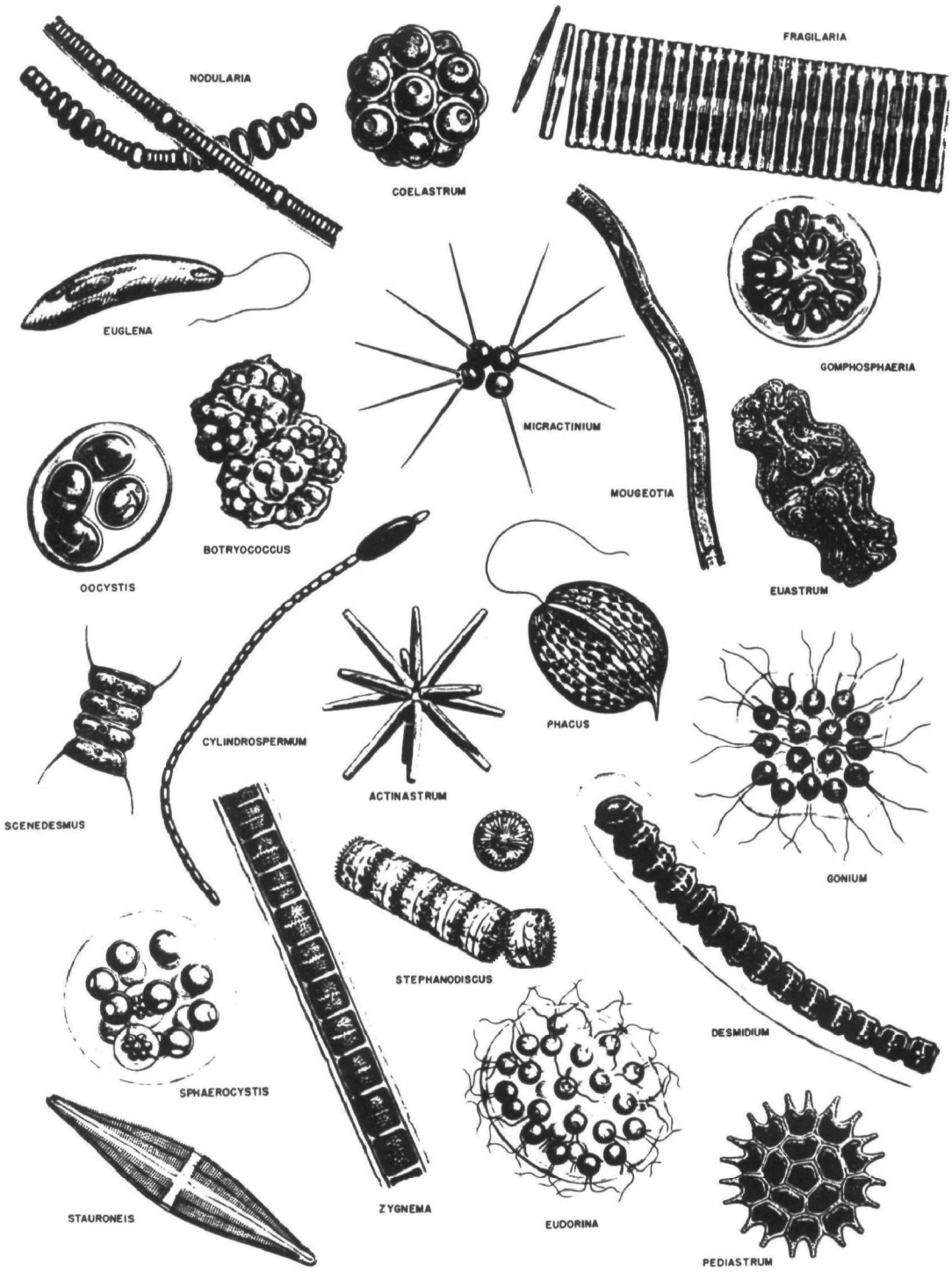

PLATE 5

ALGAE GROWING ON RESERVOIR WALLS

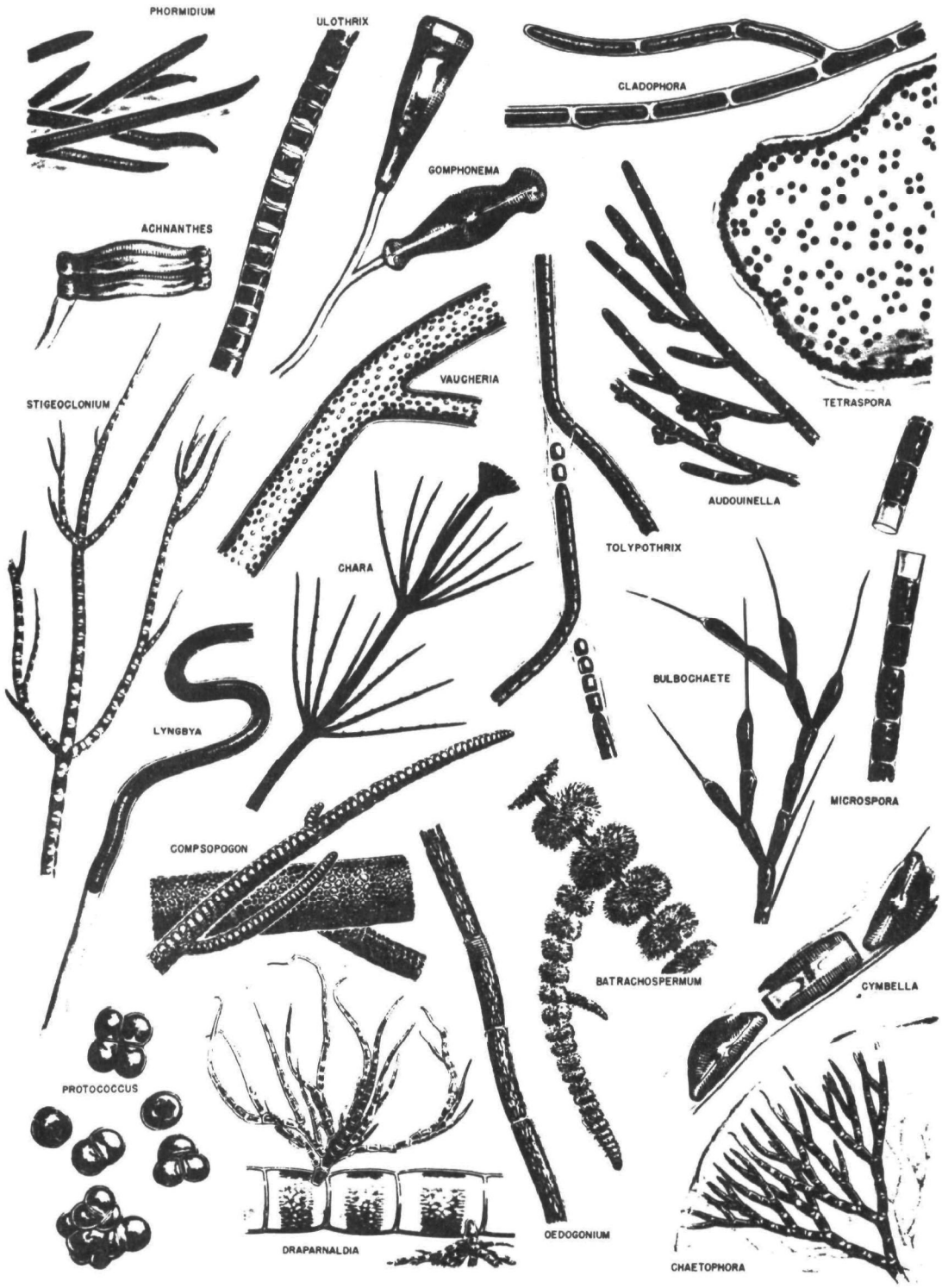

PLATE 6

ALGAE AS INDICATORS OF POLLUTION

I LIMITATIONS

A Algae are only one of a number of types of organisms present which could be considered.

B Forms recognized here as algae are comparatively simple, pigmented, aquatic organisms, including blue-greens, greens, diatoms and pigmented flagellates.

C Various pollutants react differently on algae. Organic pollutants such as household sewage will be dealt with here.

D No algae are intestinal organisms. They therefore are not indicators of pollution in the same way that coliform bacteria are.

II ALGAE AND ORGANIC POLLUTION

A Heavy pollution may tend to limit various kinds of algae to certain zones in the affected area.

B These zones are distinguished according to the degree of change which has occurred in the organic wastes. One set of names for these zones includes the Polysaprobic, alpha-mesosaprobic, beta-mesosaprobic and oligosaprobic.

C A few "pollution" algae are common in the first two zones. Many algae are common in and often limited to one or both of the last two zones.

D Some workers have listed separately those algae indicative of each of the four zones.

III REASONS FOR SELECTIVITY OF POLLUTANTS TO ALGAE

A Certain components of wastes are chemicals toxic to some algae but not to others.

B Wastes may have physical effects on certain algae. May cause plasmolysis, change in rate of absorption of nutrients, etc.

C Wastes may reduce available light, increase the water temperature, and cover up the areas for attachment to rocks.

D Wastes may prevent algal respiration at night by reducing the DO of water.

E Wastes may stimulate other organisms at the expense of certain algae.

F Products of waste decomposition may act as powerful growth stimulants for certain algae.

IV ALGAE AS INDICATORS OF POLLUTION

A Selection of list of "pollution" algae follows an evaluation of the kinds reported in published reports by numerous workers as relatively prominent in, or representative of, the polysaprobic and alpha-mesosaprobic zones in a stream polluted with sewage. It includes also other conditions or areas approximating these zones.

B A total list of more than 1000 kinds of algae has been compiled to date.

1 In order to tabulate the information, an arbitrary numerical value is allotted to each author's record of each pertinent alga.

2 The algae are then arranged in order of decreasing emphasis by the authors as a whole.

VI SOME GENERA AND SPECIES OF ALGAE HIGH ON THE LIST ARE AS FOLLOWS:

A Genera: Oscillatoria, Euglena, Navicula, Chlorella, Chlamydomonas, Nitzschia, Stigeoclonium, Phormidium, Scenedesmus, Ankistrodesmus, Phacus.

B Species: Euglena viridis, Nitzschia palea, Oscillatoria chlorina, Oscillatoria limosa, Oscillatoria tenuis Scenedesmus quadricauda, Stigeoclonium tenue, Synedra ulna and Pandorina morum.

VII SOME ALGAE REPRESENTATIVE OF CLEAN WATER ZONES IN STREAMS:

Chrysococcus rufescens, Cocconeis placentula, Entophysalis lemaniae, and Rhodomonas lacustris.

VIII RELIABILITY IN USE OF INDICATORS DEPENDS IN PART UPON ACCURATE IDENTIFICATION OF SPECIMENS

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This outline was prepared by C.M. Palmer, Aquatic Biologist, Cincinnati Water Research Laboratory, FWPCA.

POLLUTED WATER ALGAE

PLATE 3

CLEAN WATER ALGAE

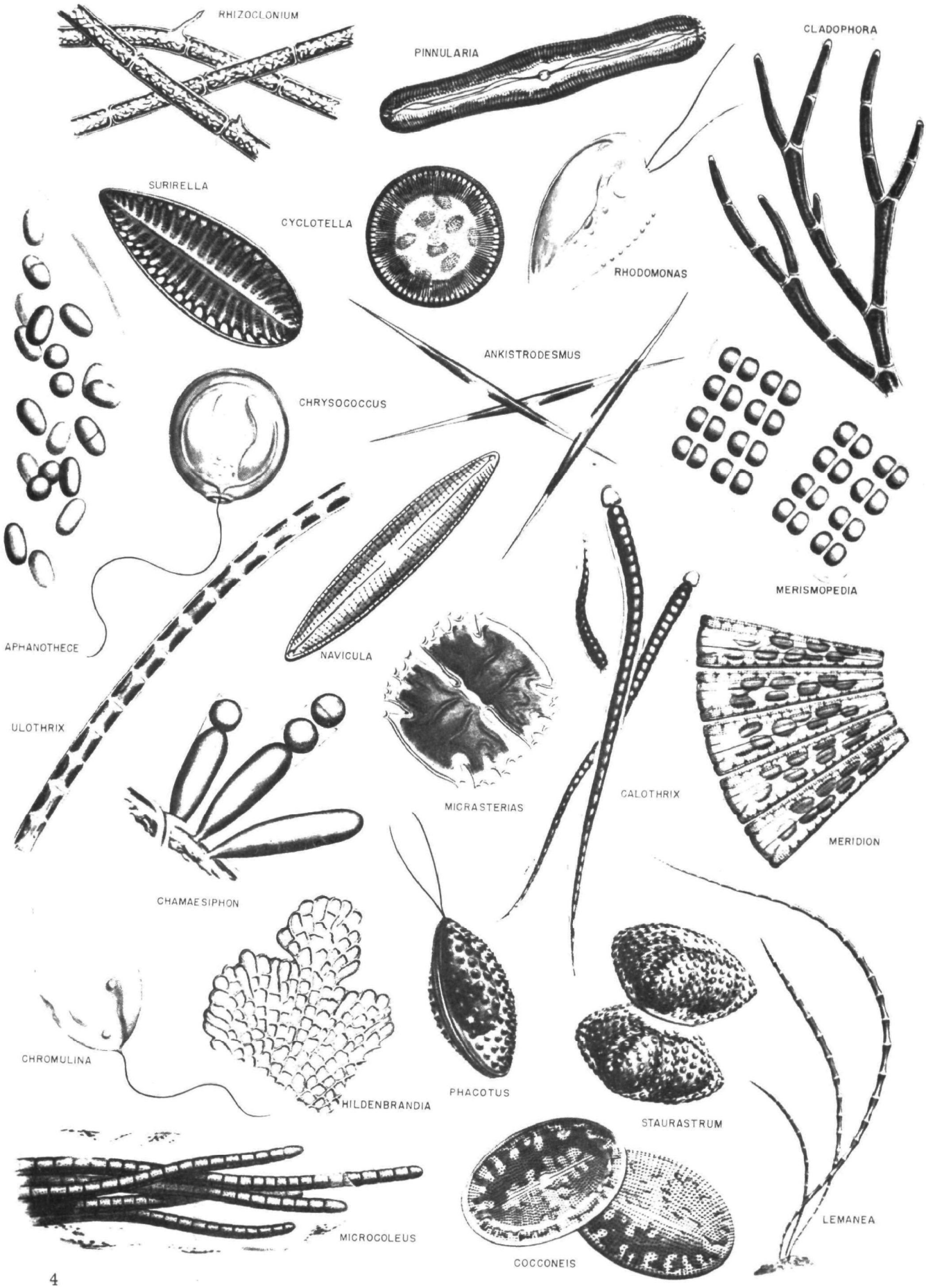

PLATE 4

PUBLIC HEALTH SIGNIFICANCE OF TOXIC ALGAE

I INTRODUCTION

A Increasing interest is being shown in toxic algae as a result of a recent increase in the number of reported cases of animal poisoning, and suspected cases of direct human poisoning thereby. Indirect poisoning of man from eating poisoned shellfish has been well demonstrated, and the poisoning of fish (for human consumption) by algae is suspected.

B Two general groups of algae are of outstanding importance:

1. The blue green algae (Cyanophyceae).

2 Certain armored flagellates (Dinophyceae).

3 Although both groups occur in both fresh and salt waters, the blue green algae alone are known to be toxic in fresh water. In marine waters, the armored flagellates predominate but other groups are also involved.

C. The biochemical nature of algae toxins is not well understood. They seem in general to be substances of low molecular weight. They are capable of producing death or illness in mammals and fish even when the algae cells themselves have been excluded.

II TOXIC FRESH WATER ALGAE

A. Blue green algae associated with toxic outbreaks include the following (not all blue green algae are toxic):

TABLE I

Toxic Fresh-Water Algae

Anabaena
Anabaena circinalis
Anabaena flos-aquae
Anabaena lemmermanni
Anacystis (Microcystis)
Anacystis cyanea (Microcystis aeruginosa)
Anacystis cyanea (Microcystis flos-aquae)
Anacystis cyanea (Microcystis toxica)
Aphanizomenon flos-aquae
Gloeotrichia echinulata
Gloeotrichia pisum
Gomphosphaeria lacustris
(Coelosphaerium kuetzingianum)
Lyngbya contorta
Nodularia spumigena
Rivularia fluitans

B Instances of animal poisoning from algae polluted waters are numerous.

1 Toxicity to domestic animals has been reported from around the world.

a First published account in 1878 reported sheep, horses, dogs, and pigs dying in South Australia after drinking water containing a heavy concentration of Nodularia spumigena.

b Arthur 1886 reported cattle in Minnesota dying within one-half hour after drinking algae polluted water.

c Hatch 1959 has reported stock killed and made ill by drinking algae polluted water from barnyard watering tanks.

d Symptoms generally included prostration and convulsions, followed by death.

2 Chickens, turkeys, ducks and geese have also been killed.

3 Dying and decomposing masses of Aphanizomenon flos-aquae have killed a variety of fish under experimental conditions.

4 Most of the outbreaks of algae poisoning have occurred during periods of continuous hot weather when the water had a high organic content, and when the algae were

concentrated at one end of a lake or pond by a gentle wind.

5 Only a single species of alga has been involved in each instance.

C Until recently, no cases of human illness or death have been reliably traced to drinking water containing toxic algae.

1 During droughts of '30s widespread gastroenteritis in several eastern cities was ascribed by some investigators to huge growths of algae in rivers used as water supplies, even though water was run through water treatment processes including heavy chlorination.

2 Recent reports from Saskatchawan indicate that heavy blue green algae growths there have seriously interferred with the recreational use of water at certain resort areas.

3 The toxic material from certain algae may survive the laboratory equivalent of water treatment using alum coagulation, filtration and chlorination. It may survive activated carbon treatment in amounts ordinarily used in water treatment plants.

III TOXIC MARINE ALGAE

A Toxic marine algae are found predominately among the armored flagellates. The greens, blue greens, and others also contribute toxic forms. The following have been listed as significant:

TABLE II

Toxic Marine Algae

Armored flagellates (Dinoflagellates)

Cochlodinium catenatum
Exuviaella baltica
Gonyaulax catenella
Gonyaulax polyedra
Gonyaulax tamarensis
Gymnodinium brevis
Gymnodinium veneficum
Gymnodinium splendens
Gymnodinium mitimoto
Pyrodinium phoneus

Flagellate

Hornellia marina
Prymnesium parvum

Brown

Egregia laevigata
Hesperophycus harveyanus
Macrocystis pyrifera
Pelvetia fastigiata

Red

Gelidium cartilagineum var. robustum

Green

Caulerpa serrulata

Blue Green

Lyngbya aestuarii
Lyngbya majuscula
Trichodesmium erythraeum

B Blooms of planktomic marine algae may be of various colors, often called "Red tide" or "Red water".

1 May or may not be toxic.

2 Toxicity may be due to a true toxic fraction or to secondary conditions such as oxygen deficiency, hydrogen sulfide liberation on decomposition, or associated bacterial pollution.

C Occurrence of Red Tides

1 Trichodesmium - widespread and recurrent patches in the Philippines, East Indian Archipelogo, and along the coast of South America.

2 Trichodesmium and dinoflagellates - Red Sea and north-west Indian Ocean.

3 Chloromonadineae (Hornellia marina) occurs off the south-east and south-west Indian Coast.

4 Chrysophysean flagellate (Prymnesium parvum) occurs in Denmark and Israeli ponds.

5 Dinoflagellates blooms associated with untoward conditions have been universally recorded from all over the world.

a Southern California - Gonyaulax polyedra and G. catenella.

b Washington State - Gymnodinium splendens.

c Florida and Gulf of Mexico - Gymnodinium brevis, and Gonyaulax catenella.

d Bay of Fundy - Gonyaulax tamarensis.

e Belgium - Pyrodinium phoneus.

f Portugal - Gonyaulax polyedra.

g Angola, West Africa - Exuviaella baltica.

h Japan - Gymnodinium mikimotoi and Cochlodinium catenatum.

i Sidney Australia - Gonyaulax polyedra.

D Toxic Dinoflagellates may be present and cause damage even though not always present in sufficient numbers to cause visible red tides.

1 Gonyaulax catenella occurs from Southern California to Alaska. It is the primary source of the poison in shellfish on the West Coast.

a Organism found in stomachs of toxic mussels in considerable numbers.

b Yearly maximun of Gonyaulax occurs during and preceding the poison in mussles.

c Mussles kept in the laboratory in clean aerated sea water lose their toxicity, whereas if Gonyaulax is present they increase in toxicity.

d An acid extract of collected organisms contains the poison.

e Organisms have been grown in pure culture and shown to contain the poison.

2 Gonyaulax Tamerensis is believed to be the cause of poisonous shellfish in the Bay of Fundy.

3 Pyrodinium phoneus is believed to be the cause of poisonous shellfish in Belgium.

4 Gymnodinium veneficum toxin has been obtained salt-free by dialysis and concentrated by evaporation under reduced pressure. Isolated from an area near Plymouth, England.

5. Gymnodinium brevis - Bacterial free cultures with concentrations from 2.3 to 4.8 million organisms per liter were toxic to three species of fish.

E Physiological Effects

1 Gonyaulax catenella toxin causes paraesthesia, loss of strength in mussels of the extremities and neck, and death by respiratory failure. Toxin apparently does not harm shellfish, but shellfish may concentrate and store the poison in their tissue. Subsequent ingestion of the shellfish by man or other animals may cause death.

2. Gymnodinium veneficium toxin may cause massive fish kills. Small fish, namely gobies, die within 10 minutes in toxic cultures. Action seems to be on the nervous system and probably is due to respiratory failure. Injection of the toxin into the dosal lymph sac of a frog has an immediate paralyzing effect.

F Ecological factors leading to bloom are not well understood. It appears that high concentrations of nutrients, particularly nitrate and phosphate must be available. Temperatures must be suitable and there must be enough phytoplankton present as an inoculum to take advantage of these conditions.

1 Oceanographic factors

a. Water temperature - Gonyaulax tamerensis has been shown to be most prevalent in the Bay of Fundy when the water temperature is between 10 and 14°C, with an optimum of 13.9°C.

b Salinity - optimum unknown but believed to be a factor.

c Nutrients - phosphates, and Vitamin B_{12} have been shown to be essential to dinoflagellate metabolism.

d Turbulence of water.

1) Upwellings of ocean currents along the California Coast, occasionally and unpredictably bring a supply of critical nutrients to the surface from abyssal depths.

2) Tidal turbulence as in the Bay of Fundy may expose geologic deposits of nutrients.

3) Heavy rains on land have washed nutrient materials into the sea and caused blooms in Florida.

2 Small amounts of sewage pollution also may favor growth by contributing nutrient material.

3 Associated phenomena

a Extraordinary wide and rapid variations in the dissolved oxygen content of the water have been observed during and immediately following heavy concentrations of Gonyaulax.

b Biochemical oxygen demand when Gonyaulax are present is far in excess of what might be explained by sewage or other organic pollutants.

c Dinoflagellate blooms are often succeeded by blooms of organisms which prey on them. In the Bay of Fundy the ciliate protozoa, Fevella, ebrenbergii was found feeding upon Gonyaulax.

d Other organisms such as diatoms may compete with Gonyaulax for food or light and check their growth.

G Some of the complexities of dinoflagellate ecology are suggested by the epidemiology of fish poisoning suffered by people throughout tropical regions.

1 It has long been known that some species of coral are crammed with zooxanthelae symbiotic algae whose role in the corals economy is still obscure.

a The dinoflagellate Gymnodinium adriaticum has recently been isolated from a jellyfish, some sea animones and corals, G. adriaticum is not toxic but

other local species may be.

2 The parasitic dinoflagellate Odinum which lives on fish gills or skin should also not be overlooked.

3 Coral animals and shellfish which feed upon free swimming dinoflagellates could also pass their poison up the food chain to fish and predators which feed on fish.

4 Lyngbya and a few other algae also have been suggested as a cause of fish poisoning.

5 It has been suggested that there are as many as eight different kinds of fish poisoning, but only two or three of these have been studied sufficiently to indicate their similarity or dissimilarity.

a Puffer fish poisoning - very similar to shellfish poison but not identical. Symptons most frequently develop in 10 to 45 minutes and include paresthesia, hypersalivation, sub-normal temperature, decreased blood pressure and a weak pulse. The paresthesia gradually develops into severe numbness and finally terminates in extensive muscular paralysis. If death occurs, it is generally within 4 to 24 hours and results from a progressive ascending paralysis involving the respiratory muscles.

b Ciguatera poisoning - barracuda, snappers and various reef fish are most commonly incriminated. The poison is fat soluble and on this basis appears to be distinct from shellfish poison or puffer fish poison. Symptoms develop in from 1 to 6 hours and include paresthesia, headache, fever, profuse sweating, rapid weak pulse, prostration, muscle pains, and joint aches. Initial symptoms sometimes may consist of nausea, vomiting and diarrhea, and in severe intoxications, paradoxical sensory disturbances may be present in which hot objects may feel cold. Death may occur within a few minutes but generally requires several days. Muscular twitchings, tremors and convulsions are followed by death due to respiratory failure.

c Gymnothorax poisoning - frequently caused by consuming moray eels. As with ciguatera and puffer poisoning, the initial symptoms include tingling and numbness about the lips, tongue, hands and feet, sometimes followed by nausea, vomiting, a metallic taste, diarrhea and abdominal pain as in ciguatera. The characteristic signs, however, appear to be profuse perspiration, excessive mucus production, rapid respiration, high fever, purposeless movements and violent convulsions. In severe intoxication, death does not occur until after 14 to 25 days.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226. Portions of this outline were taken from previous training outlines by W. M. Ingram and E. F. McFarren.

ORGANIC ENRICHMENT AND DISSOLVED OXYGEN RELATIONSHIPS IN WATER

I INTRODUCTION

A Oxygen normally composes approximately 20% of the atmosphere, but from only 0.5 to 1% of unpolluted water by volume (9 - 14 milligrams per liter, or parts per million).

B Physically, oxygen is a colorless gas, responsive to the laws of solubility, temperature, etc.

C The amount of oxygen present can be readily determined by various methods, both chemical and physical.

D Biologically, oxygen is one of the key elements to all life, all living organisms contain it along with hydrogen and carbon. It is the physiological "complement" of carbon dioxide.

E When properly interpreted, oxygen concentration can serve as an excellent index to water quality.

1 It is widely present.

2 It is intimately involved in many processes, chemical, physical, and biological.

II BIOLOGICAL OR PHYSIOLOGICAL RELATIONSHIPS (Figure 1)

A Photosynthesis is the biological starting point for synthesizing living substance out of non-living carbon dioxide, water, and other materials. In the process, radiant energy is adsorbed from the sun (hence it is an endothermic reaction) and free molecular oxygen is released to the environment as long as sufficient light is available, i.e., during daylight only.

1 The conventional empirical formula for photosynthesis is a very great simplification, as many as twenty steps may actually be involved (like respiration, controlled by systems of enzymes).

$$6\ CO_2 + 6\ H_2O + 673\ Cal$$

(Energy sunlight)

↓ chlorophyll (enzyme system)

$$C_6H_{12}O_6 + 6\ O_2$$

(Simple sugar)

2 The end product is a basic organic material known as a simple sugar (carbohydrate, monosaccharide, of which "glucose" is an example, see Figures 1, A) which serves as a starting point for the elaboration of higher organic substances, and which may also be oxidized or "respired" by the cell to release the stored chemical energy.

3 Synthesis of organic materials (living protoplasm) from simple sugars and other substances in bacteria, algae, and man.

a Glucose and other simple sugar molecules can be combined by living organisms such as algae into other carbohydrates such as starch.

b After modification into fatty acids and glycerol, fats can be made.

c Combination with nitrogen and other minerals taken in from the surrounding water produces amino acids. These can then be combined into proteins, the most complicated of all life substances. Plants are the only organisms that can synthesize amino acids completely "from scratch."

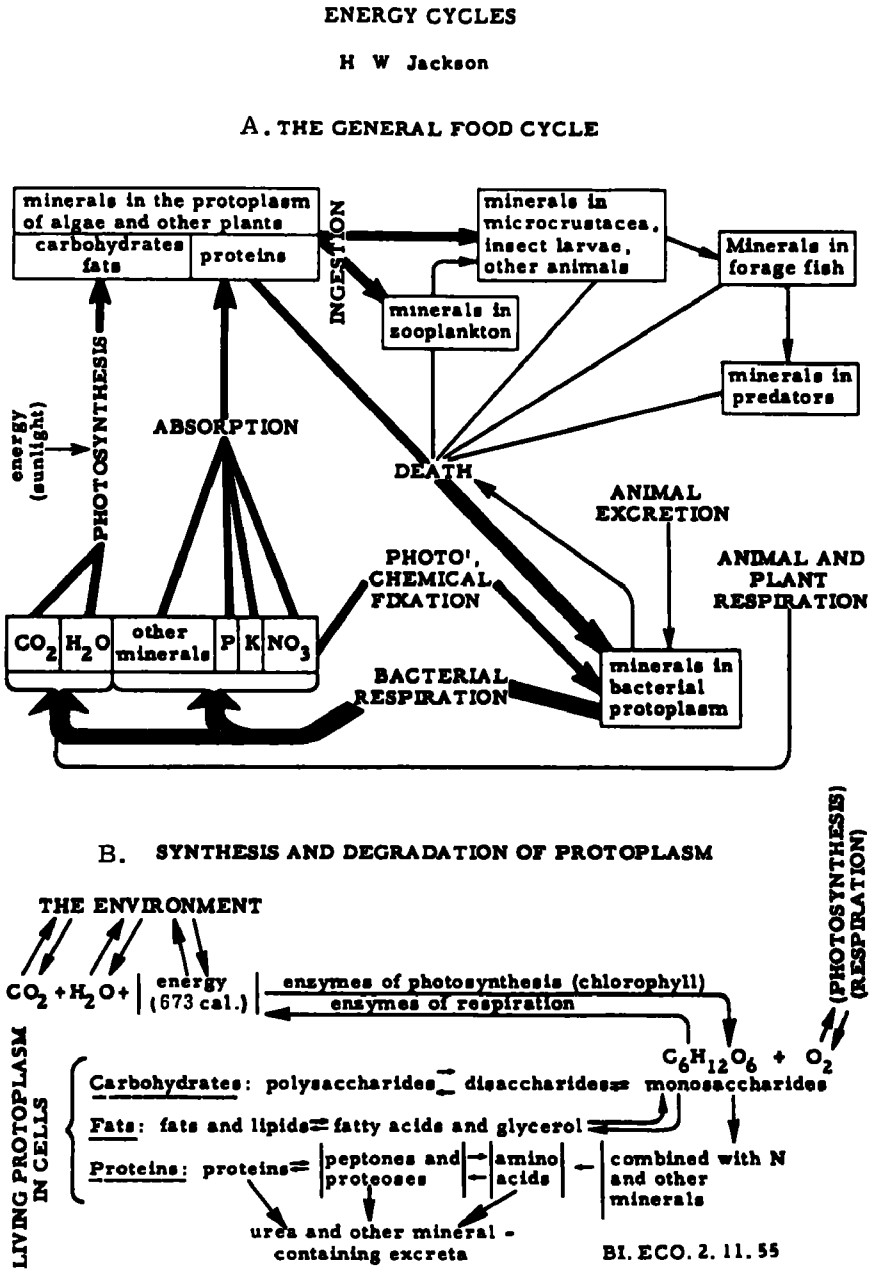

ENERGY CYCLES
H W Jackson
A. THE GENERAL FOOD CYCLE
minerals in the protoplasm of algae and other plants
carbohydrates fats
proteins
INGESTION
minerals in microcrustacea, insect larvae, other animals
Minerals in forage fish
minerals in zooplankton
minerals in predators
energy (sunlight)
PHOTOSYNTHESIS
ABSORPTION
DEATH
ANIMAL EXCRETION
PHOTO', CHEMICAL FIXATION
ANIMAL AND PLANT RESPIRATION
CO2
H2O
other minerals
P
K
NO3
BACTERIAL RESPIRATION
minerals in bacterial protoplasm
B. SYNTHESIS AND DEGRADATION OF PROTOPLASM
THE ENVIRONMENT
CO2 + H2O +
energy (673 cal.)
enzymes of photosynthesis (chlorophyll)
enzymes of respiration
(PHOTOSYNTHESIS) (RESPIRATION)
C6H12O6 + O2
LIVING PROTOPLASM IN CELLS
Carbohydrates: polysaccharides disaccharides monosaccharides
Fats: fats and lipids fatty acids and glycerol
Proteins: proteins peptones and proteoses amino acids combined with N and other minerals
urea and other mineral - containing excreta
BI. ECO. 2. 11. 55

Figure 1

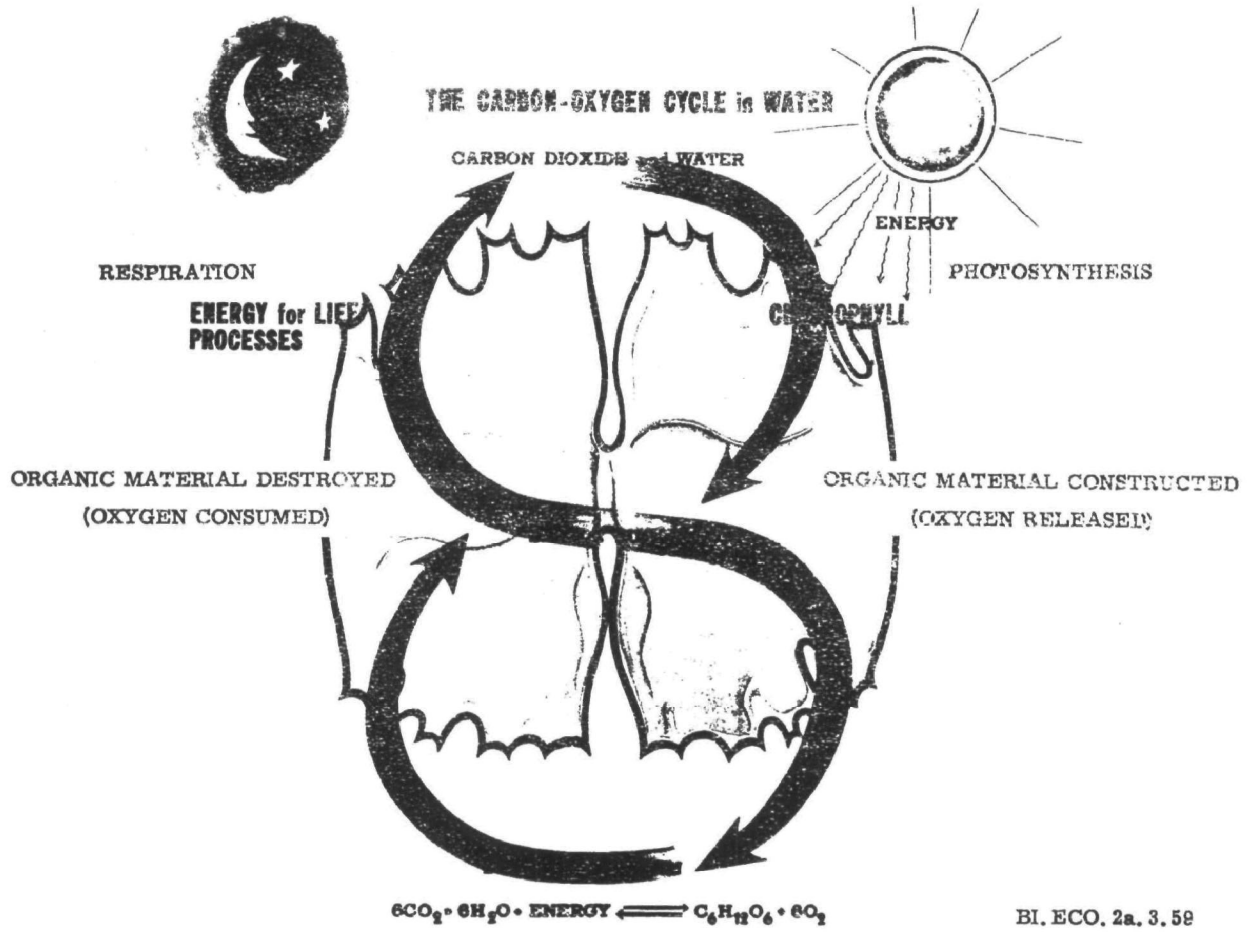


Figure 2

4 All of these synthetic or growth processes are endothermic and require energy to consummate. This energy is obtained by respiration.

B Respiration. Life processes such as those mentioned previously (II A 3, also physical movement) are energized by the controlled oxidation or "respiration" of single sugars. This is a cellular or physiological process and continues constantly in all living things, independently of light, as long as life persists.

1 Types of respiration. Respiration is an energy releasing or exothermic biochemical process and is to be distinguished from the external bodily act of "breathing", which is a process involving a lung, gill, or other organ, whereby molecular oxygen is brought inside the body of the organism and carbon dioxide is released. Breathing is transportation; respiration is burning or biochemical utilization.

a Aerobic respiration. The most common form of respiration is illustrated by the oxidation of glucose, using free dissolved oxygen from the environment. This is also the most efficient type since virtually all of the chemical energy contained in the glucose molecule is released (673 Cal.) and the residue is completely mineralized or stabilized to carbon dioxide and water.

Although actually taking place in more than twenty steps involving as many different enzymes, the overall process can be empirically represented by the following formula:

$C_6H_{12}O_6 + 6\,O_2$

(a simple sugar such as glucose)

$\downarrow$

$6\,CO_2 + 6\,H_2O + 673$ Cal

(enzymes) (energy)

b Anaerobic respiration. If free dissolved oxygen (DO) is not available from the environment, certain types of bacteria, fungi, and other organisms known as "facultative aerobes" can switch to another, less efficient type of respiration and still obtain energy by breaking down simple sugars. This is known as anaerobic respiration. Some types of microorganisms, the true anaerobes, can thrive only under anaerobic conditions.

The anaerobic respiration of glucose involves splitting the molecule without the use of outside oxygen so as to release some of the contained energy, but not all. The remainder is still available in the end products. A common empirical relationship is as follows:

$3\,C_3H_{12}O_6 \longrightarrow 2\,CH_3(COOH)_2$

(glucose) (enzymes) (lactic acid)

$+ 2\,CH_3CH_2OH + 2\,CH_3\,COOH$

(ethyl alcohol) (acetic acid)

$+ \underset{\text{(energy)}}{59\ 1/2\ \text{Cal}}$

c Anaerobic respiration of proteins and their derivations by microorganisms (known as putrefaction) often leads to the production of foul smelling end products. Aerobic respiration of proteins (known as decay) seldom produces offensive odors. In addition to carbohydrates, proteins, fats, and various other natural and unnatural hydrocarbons, and other substances can also be broken down or respired to relatively stable end products by microorganisms.

2 The adaptability of life is remarkable. By the same mechanisms of genetics and natural selection which have resulted in the evolution of present day life on earth, microorganisms have evolved which are capable of obtaining energy from, or in other words, "respiring" a great variety of new synthetic materials, the products of modern industry. This is the basis for the biological treatment of wastes.

a The types of biological mechanisms involved, in general, are known and to a degree, understood.

b Many species or kinds of organisms may be involved in the stabilization of a single type of waste.

3 Significance of respiration in sanitary engineering. The process of respiration uses or "demands" oxygen from the environment. Any material, such as sewage, an organic industrial waste, motor oil, etc., which will support life of any kind will thus exert (through the organisms living on it) a "BOD".

a Respiration has two end "products": energy, by which the organisms live; and degraded, mineralized, or stabilized material such as digested sewage sludge which retains little or no energy available to organisms. That is, they have no biochemical oxygen demand or BOD remaining.

b Since it is independent of light, it goes on night and day.

III PHYSICAL PHENOMENA

A Solubility of oxygen in pure water varies with temperature and pressure according to well known laws and values.

1 We expect to find less oxygen in warmer waters and more in colder. This has both seasonal and geographical implications.

2 Dissolved substances such as salts tend to reduce the solubility of oxygen at a given temperature (See Figure 3 - Oxygen Solubility at Selected Salinities).

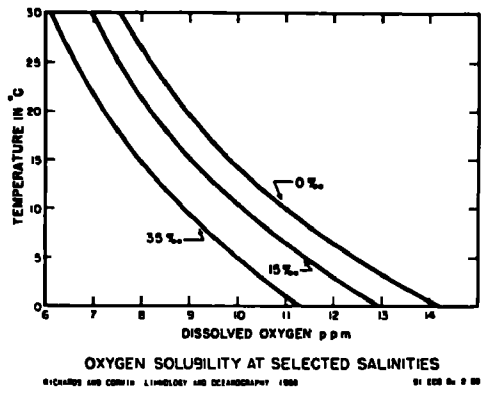


Figure 3

3 The concentration of oxygen often is expressed as percent of saturation.

a Organisms, however, are affected by the real quantities present, and actually demand the most oxygen at the highest temperature, when the solubility is lowest.

b It is therefore more significant, where organisms and stream conditions are concerned, to express concentration as milligrams per liter (or parts per million).

B The movement or distribution of oxygen throughout a water mass is not entirely dependent on molecular diffusion, but also involves various types of gross water movements. Reaeration (or aeration) is the transfer of oxygen from the air into the water mass.

IV CHEMICAL FACTORS

A Chemical toxicity may delay the exertion of biological oxygen demands, or the release of oxygen by biological mechanisms.

B Chemical substances may themselves demand oxygen from the water.

1 If oxygen is available, the chemical oxygen demands tend to be satisfied relatively quickly and hence are likely to be a more local problem.

2 They are independent of any biological effect.

3 They are in addition to biological action.

V OXYGEN AND EUTROPHICATION

A Diurnal interactions of photosynthesis and respiration. (Figure 6)

1 Life, growth, and hence the need for oxygen for respiration continue twenty-four hours a day, as long as life persists.

2 Since photosynthesis in nature is activated by radiant energy from the sun, it is operative only during the daytime. It releases approximately twenty times as much oxygen as is consumed in cellular respiration, however, so there is a great excess left over which diffuses out into the surrounding water. Here it is available to biochemical oxidative demands of other organisms with the result that the ecological system becomes "aerobic."

3 DO's affected by photosynthesis thus tend to be highest in the daytime (particularly mid-afternoon) and lowest at night (3 - 4 A.M.).

B Organic materials from domestic sewage or similar wastes are readily available to microorganisms. Assimilation of food, and growth and multiplication of the population can thus begin relatively quickly, thus establishing a demand for oxygen for respiration.

1 It takes a significant period of time for a population of bacteria, fungi, and other microorganisms such as protozoa to become established, after the initiation of growth of the few original organisms, even under the best of conditions.

2 Dissolved oxygen does not thus disappear instantly from the water upon the admixture of sewage, but rather begins to diminish slowly. As the population of oxygen consuming organisms builds up, the deficit in the water increases. As this occurs, any equilibrium which may have existed with the atmosphere is destroyed, and the rate of aeration through the surface film increases.

a As the population of microorganisms grows still larger, its rate of increase begins to increase (logarithmic growth). Very soon a size of population is achieved where the demand for oxygen exceeds the amount which can be supplied through the surface film. The concentration of free DO now begins to drop sharply and may go to zero.

b If sufficient food for the microorganisms remains, anaerobic conditions will now prevail. Oxygen, of course, continues to enter at a maximum rate through the surface film, but is immediately used up. The overall rate of oxidation of the waste or food material however, is now very low (although the total amount may be large), as surface aeration can supply but a small portion of the oxygen needed.

c If there is no replenishment of the food supply by repollution, the big population of fungi and bacteria eventually uses up most of the available substrate or food, and begins to starve. Growth now begins to slow down, and the population eventually drops back to its original low level.

d Meanwhile time has passed. An abundance of fundamental plant nutrients such as nitrate, phosphate, potash, etc., have been released to the water as a result of microbial respiration. Algae begin to grow on this substrate, and increase rapidly to tremendous numbers.

e Algal photosynthesis thus suddenly begins to release great quantities of oxygen in the daytime. At first this is exhausted each night by respiration of the algae and other microorganisms, but soon persists around the clock until the algae in turn have exhausted their food supply and return to numbers normal for an unpolluted stream.

f With the above mentioned advent of free DO from the algae, the more efficient aerobic type of respiration can again be employed by the microorganisms. This hastens the oxidation or stabilization process, and leaves behind but a minimum residue of well mineralized material to accumulate on the bottom.

g It should be mentioned that turbidity from suspended microorganisms and other organic solids frequently inhibit the establishment of an algal population until the biochemical oxidation or stabilization process has been well started. Turbidity inhibits algal growth primarily by shading and suppression of photosynthesis.

C When organic material is dumped into a body of water faster than it can be oxidized by the mechanism described above, it tends to accumulate on the bottom in a partially digested condition known as sludge.

1 Great quantities of nutrient energy are still present, as well as a huge population of microorganisms. There is a very great need for oxygen which, if available, is avidly taken up by the organisms in the surface layers.

a Aerobic conditions in the overlying waters thus tend to hasten the stabilization of sludge banks by providing oxygen for aerobic type respiration.

b Anaerobic waters, on the other hand, tend to preserve the sludge by restricting all organisms to anaerobic types of respiration. It has been reported that the speed of decomposition begins to be restricted at concentrations below 1 ppm.

2 Anaerobic conditions are present throughout the sludge mass below the surface. Stabilization is still proceeding, but at a much slower rate than if oxygen were present.

D In the deeper reservoirs, lakes, rivers, and estuaries thermal stratification may develop in the summer.

1 The epilimnion or upper layer, being in contact with the atmosphere, receives a continual replenishment of oxygen by aeration processes.

2 If turbidity is not excessive, photosynthesis by algae (Figure 5) will also provide oxygen.

3 The hypolimnion or lower layer, being separated from the atmosphere and frequently from the light, tends to acquire an excess of carbon dioxide and a deficiency of oxygen due to the respiratory activities of microorganisms (such as protozoa, fungi, and bacteria). (Figure 4)
This effect is heightened if the bottom material is high in organic content as has been shown below.

Figure 4
Non-Pigmented, Non-Oxygen-Producing, Protozoan Flagellates

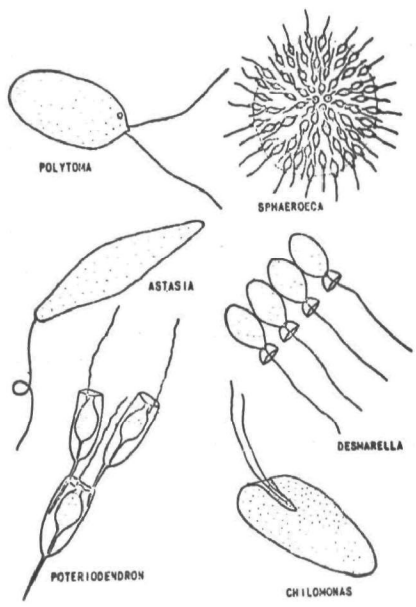
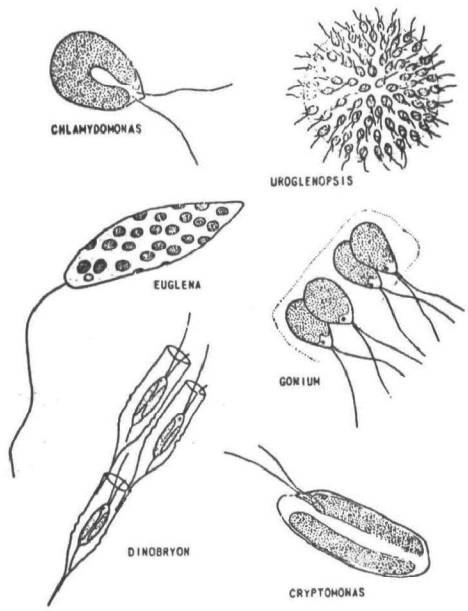


Figure 5
Pigmented, Oxygen-Producing, Algal Flagellates



VI PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION (Figures 4, 5, 6)

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

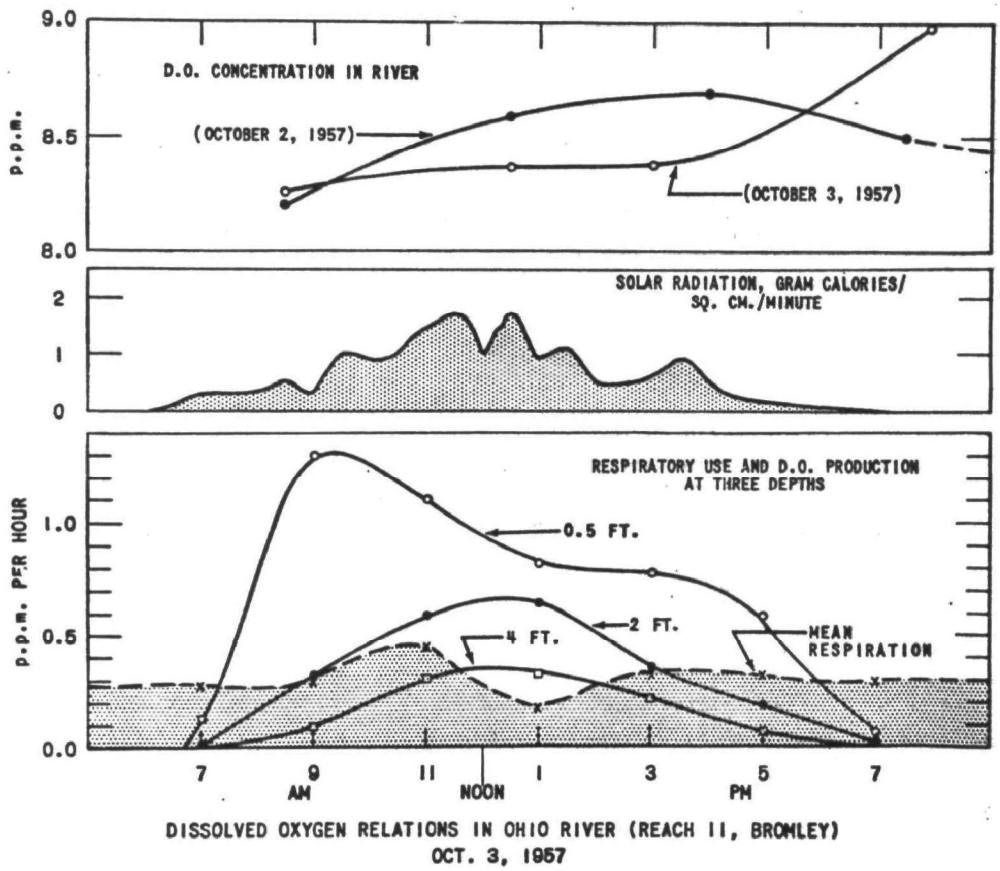
1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

3 Death of large algal populations may lead to obnoxious odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge in estuaries and back waters may be considerable.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels: (Figure 7)

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.



DISSOLVED OXYGEN RELATIONS IN OHIO RIVER (REACH II, BROMLEY)
OCT. 3, 1957

Figure 6

Mean Surface Water Productivity Rates of the Great Lakes and Other Freshwater Lakes and Ocean Areas.

Lake or Ocean and Location	Productivity Rate in mg.C/ m^3 /day	Investigator
Lake Superior	16.62	Parkos (1967-68)
Lake Huron	23.04	Parkos (1968)
Lake Michigan	37.62	Parkos (1967)
Lake Erie	175.20	Parkos (1968)
Grand Traverse Bay, N.E. Lake Michigan	0.34	Saunders et al. (1962)
Douglas Lake, Mich.	0.23	Saunders et al. (1962)
Bras d'Or Lake, Nova Scotia, Canada	23.5	Geen and Hargrave (1966)
Brooks Lake, Alaska	3.2	Goldman (1960)
Naknek Lake, Alaska	8.8	Goldman (1960)
Torne Träsk, Sweden	6.4	Rodhe (1958b)
Ransaren, Sweden	8.8	Rodhe (1958b)
Lake Erken, Sweden	221.0	Rodhe (1958b)
Lake Esrom, Denmark	1600	Jonasson and Mathiesen (1959)
Lake Furesø, Denmark	2100	Jonasson and Mathiesen (1959)
N.E. Atlantic - near Denmark	21	Steemann-Nielsen (1960)
North Atlantic, same lat. as Great Lakes	7.5	Steemann-Nielsen (1958b)
Southeast Pacific	3.3	Holmes (1961)
Southwest Pacific	7.2	Angot (1961)
North Pacific, Sub-Arctic Region	6.4	Koblentz-Mishke (1961)

Figure 7

1 Earlier notation cited the release of oxygen during utilization of CO_2 during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.

2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

3 Photosynthesis tends to occur at the surface where light intensity is greatest (Figure 6). Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.

ACKNOWLEDGEMENTS:

This outline contains certain material submitted by F.J. Ludzack and M.E. Bender.

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This outline was prepared by H.W. Jackson, Chief Biologist, National Training Center, FWPCA, Cincinnati, OH 45226.

PLANKTON IN OLIGOTROPHIC LAKES

I INTRODUCTION

The term oligotrophic was taken from the Greek words oligos -- small and trophein -- to nourish, meaning poor in nutrients. Lakes with low nutrient levels have low standing crops of plankton. The term is now commonly applied to any water which has a low productivity, regardless of the reason.

II PHYSICAL AND CHEMICAL CHARACTERISTICS OF OLIGOTROPHIC LAKES*

A Very deep; high volume to surface ratio

B Thermal stratification common; volume of the hypolimnium large compared to the volume of the epilimnion

C Maximum surface temperature rarely greater than 15°C

D Low concentrations of dissolved minerals and organic matter.

1 Phosphorus, less than 1 microgram per liter

2 NO_3-Nitrogen, less than 200 micrograms per liter

E Dissolved oxygen near saturation from surface to bottom

F Water very transparent, Secchi disk readings of 20-40 meters are common

G Color dark blue, blue-green, or green

III PLANKTON

A Quantity

1 Standing crop very low

a Ash-free weight of plankton, less than 0.1 mg per liter (compared to 1 mg per liter or more in eutrophic lakes).

b Chlorophyll, 1 mg per M^3 or less

c Cells counts, less than 500 per ml

2 Zooplankton to phytoplankton volume ratio, 19:1.

B Quality

1 European biologists have found oligotrophic lakes to be dominated by Chlorophyta (usually desmids), chrysophyta (such as Dinobryon), and Diatomaceae (Cyclotella and Tabellaria). Eutrophic lakes are dominated by Synedea, Fragilaria, Asterionella, Melosira, blue-green algae, Ceratium, and Pediastrum. Nygaard devised several phytoplankton quotients based on these relationships

a Simple quotient

$$\frac{\text{Number of species of Chlorococcales}}{\text{Desmidiaceae}} = \begin{array}{l}\text{if } <1, \text{ oligotrophic} \\ \text{if } >1, \text{ eutrophic}\end{array}$$

b Compound index

$$\frac{\text{Myxophyceae+Chlorococcales+Centrales+Euglenaceae}}{\text{Desmidiaceae}} =$$

if <1, oligotrophic

if 1-2.5, mesotrophic

if >2.5, eutrophic

c Diatom quotient

$$\frac{\text{Centrales}}{\text{Pennales}} = \begin{array}{l}\text{if } 0\text{-}0.2, \text{ oligotrophic} \\ \text{if } 0.2\text{-}3.0, \text{ eutrophic}\end{array}$$

2 Several lists of trophic indicators have been published:

Two are listed here

	Teiling, Swedish Lakes		Rawson, Canadian Lakes
Oligotrophic	Tabelleria flocculosa	Oligotrophic	Asterionella formosa
	Dactylococcopsis ellipsoideus		Melosira islandica*
			Tabellaria fenestrata
			Tabellaria flocculosa
Mesotrophic	Kirchneriella lunaris		Dinobryon divergens
	Tetraeadon spp.		Fragilaria capucina
	Pediastrum spp.		Stephanodiscus niagarae
	Fragilaria crotonensis		Staurastrum spp.
	Attheya zachariasii		Melosira granulata
	Melosira granulata		
		Mesotrophic	Fragilaria crotonensis
Eutrophic	Aphanizomenon spp.		Ceratium hirundinella
	Anabaena flos-aquae		Pediastrum boryanum
	Anabaena circinalis		Pediastrum duplex
			Coelosphaerium naegelianum
Pronounced Eutrophy	Microcystis aeruginosa		Anabaena spp.
	Microcystis viridis		Aphanizomenon flos-aquae
			Microcystis aeruginosa
		Eutrophic	Microcystis flos-aquae

Some discrepancies can be seen in the ranking of species in the lists. These may be the result of true differences in the composition of the plankton, or may be only apparent differences which resulted from different sampling methods. Many studies (e.g. those by Hilliard, Olive, and Rawson) have been based on netted samples, which may be highly biased because they contain little of the nannoplankton. Also, it is not uncommon to characterize populations on the basis of one or two samples collected during the summer months.

3 The dominant plankton in four oligotrophic North American lakes are listed below. The Great Slave Lake and Karluk Lake data are from netted samples taken during the summer, and monthly, respectively. The Lake Superior and Lake Tahoe data are from grab samples taken twice monthly, and quarterly, respectively.

The dominant diatoms are generally similar in the four lakes. Asterionella formosa and Fragilaria crotonensis are common to all. There are also some obvious differences. Melosira islandica, the dominant diatom in the Great Slave Lake and Lake Superior, is absent from Lake Tahoe and Karluk Lake. It was not found in Crater Lake by Sovereign (1958), in the Mountain lakes of Colorado by Olive (1955) or Brinley (1950), and does not occur in WPSS samples in streams west of the Great Lakes. Tabellaria is also absent from Lake Tahoe. It was reported in Colorado lakes by Olive, but was not abundant. Brinley makes no reference to it, and Sovereign indicated that it was rare in Crater Lake samples. It is apparent that the absence of these two diatoms from Lake Tahoe is not related to the lake.

Except for the absence of Keratella cochlearis from Lake Tahoe, the rotifer populations are very similar. Data on other segments of the zooplankton population are insufficient to permit comparison.

	Rawson, Great Slave Lake	USPHS, Lake Superior	Hilliard, Karluk Lake	WPSS, Lake Tahoe
Dominant Phytoplankton	Melosira islandica Asterionella formosa Dinobryon divergens Ceratium hirundinella Pediastrum boryanum Tabellaria fenestrata Cyclotella meneghiniana Fragilaria crotonensis Fragilaria capucina Synedra ulna Eunotia lunaris	Melosira islandica Tabellaria fenestrata Cyclotella kutzingiana Melosira granulata Melosira ambigua Asterionella formosa Synedra nana Scenedesmus spp Ankistrodesmus spp. Dictyosphaerium spp.	Asterionella formosa Tabellaria flocculosa Fragilaria crotonensis Cyclotella bodanica Cymbella turgida Dictyosphaerium spp. Sphaerocystis spp Staurastrum spp.	Fragilaria crotonensis Synedra nana Fragilaria construens Fragilaria pinnata Nitzschia acicularis Asterionella formosa
Dominant Zooplankton	Keratella cochlearis Kellicottia longispina Diaptomus tenuicaudatus Limnocalanus macrurus Senecella calanoides Daphnia longispina Bosmina obtusirostris	Keratella cochlearis Kellicottia longispina	Not reported	Kellicottia longispina Daphnia spp. Diaptomus tyrelli Epischura nevadensis

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This outline was prepared by C.I. Weber, Chief, Biological Methods Section, Analytical Quality Control Laboratory, FWPCA, 1014 Broadway, Cincinnati, OH 45202.

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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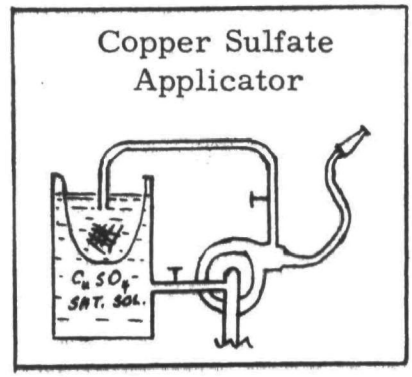

CHAPTER V

PLANKTON CONTROL

Control of Plankton in Surface Waters 3

Control of Interference Organisms in Water Supplies 4

Nutrients: The Basis of Productivity 5

CONTROL OF PLANKTON IN SURFACE WATERS

I PHILOSOPHICAL CONSIDERATIONS

A Plankton growths are as natural to aquatic areas as green plants are to land areas and respond to the same stimuli.

B Man is currently harnessing plankton forms to accomplish useful work.

1 For generation of oxygen

a Stabilization of waste waters in oxidation ponds

b Oxygen recovery from CO_2 in space travel

2 For augmentation of food supply

a Fish ponds

b Nitrogen fixation in rice growing

c Harvesting of algae for direct use as food

C A growing knowledge of the nutrient requirements of plankton organisms will lead to a more enlightened approach to ways and means of controlling their growth when desirable.

II CLASSICAL METHODS OF CONTROL

A Chemical

1 Inorganic

a Copper sulfate is used most extensively. It is most effective in preventive rather than curative treatment. It has long lasting effects in soft waters but is short-lived in hard waters due to precipitation of the Cu^{++} as a basic carbonate The precipitated material accumulates in bottom muds and is toxic to certain benthal forms, some of which serve as important fish food.

Dosages are normally based on the alkalinity of the water. When alkalinity is < 40 mg/l, the recommended dosage is 0.3 mg/l of $CuSO_4$ $5H_2O$ in total volume of water. When alkalinity is > 40 mg/l, recommended dosage is 2.0 mg/l in surface foot of water.

b Chlorine is preferable to copper sulfate in the control of certain forms of algae. However, it is difficult to apply in most instances and is very short-lived due to photo catalytic decomposition of HClO → HCl + O

2 Organic - Numerous organic compounds have been evaluated, especially in relation to control of blue-green algae. "Phygon", 2,3-dichloronaphthoquinone, has been field tested but is too specific in its action for general application.

III ECOLOGICAL CONTROL

A Theory - Ecological control is based upon the principle of preventing or restricting growth by limiting one or more of the essential requirements. This is an application of Liebig's Law of the Minimum. The logical avenues of control are as follows:

1 Elimination of light

2 Limiting nutrient materials

B Light - Many cities have solved the problem of plankton growths by the use of covered reservoirs, underground and elevated. Concurrently, they have solved contamination problems created by birds and atmospheric fallout In open reservoirs,

some success has been obtained by limiting light through the use of a film of activated carbon.

C Nutrients - Since phytoplankton (algae) serve as the base of the food chain, knowledge concerning their nutrient requirements is required for ecological control, when limitation of light is impractical. The nutrient requirements of phytoplankton are as follows:

1 Nature of - The major nutrients are:

a Carbon dioxide

b Nitrogen - ammonia and nitrates (also N_2)

c Phosphorus - phosphates.

Minor nutrients are:

d Sulfur - sulfates

e Potassium

f Trace inorganics - magnesium, iron, etc.

g Trace organics-vitamins, amino acids

2 Sources of - See Fig. 1

a Atmosphere

b Groundwater - springs

c Storm water or surface runoff

d Waste waters - domestic sewage and industrial wastes.

3 Significance of each major nutrient

a Carbon dioxide - See Fig 2
Usually present in great abundance. Rapidly replenished from atmosphere and bacterial decomposition of organic matter. No reasonable possibility of human control. Nature, however, does provide some control through elevated pH levels if carbon dioxide becomes depleted rapidly.

b Nitrogen - Like land plants, certain algal forms prefer nitrogen in the form of $NH_3(NH_4^+)$ and others prefer it in the form of NO_3^-. Both forms often become depleted during the growing season and reach maximum concentrations during the winter season. A level of 0.30 mg/l of inorganic nitrogen at the time of the spring turnover is considered to be the maximum permissible level.

All natural surface waters are saturated with nitrogen gas. This serves as a source of nitrogen for bacteria and algae capable of fixing it.

c Phosphorus - A key element in all plant and animal nutrition. The critical level is considered to be 0 01 mg/l at the time of the spring turnover. Phosphorus is needed to sustain nitrogen fixing forms.

D Practice Of

1 Exclusion of light - Practice well established in distribution system reservoirs but impractical on large storage reservoirs.

2 Nutrient limitation

a Control of surface run-off quality

1) Agricultural
2) Other

b Diversion of sewage plant effluents

1) Madison, Wisconsin
2) Detroit Lakes, Minnesota
3) Pending - State College, Pa.

c Tertiary treatment of sewage

1) Nitrogen removal - Because of the several forms is very difficult.

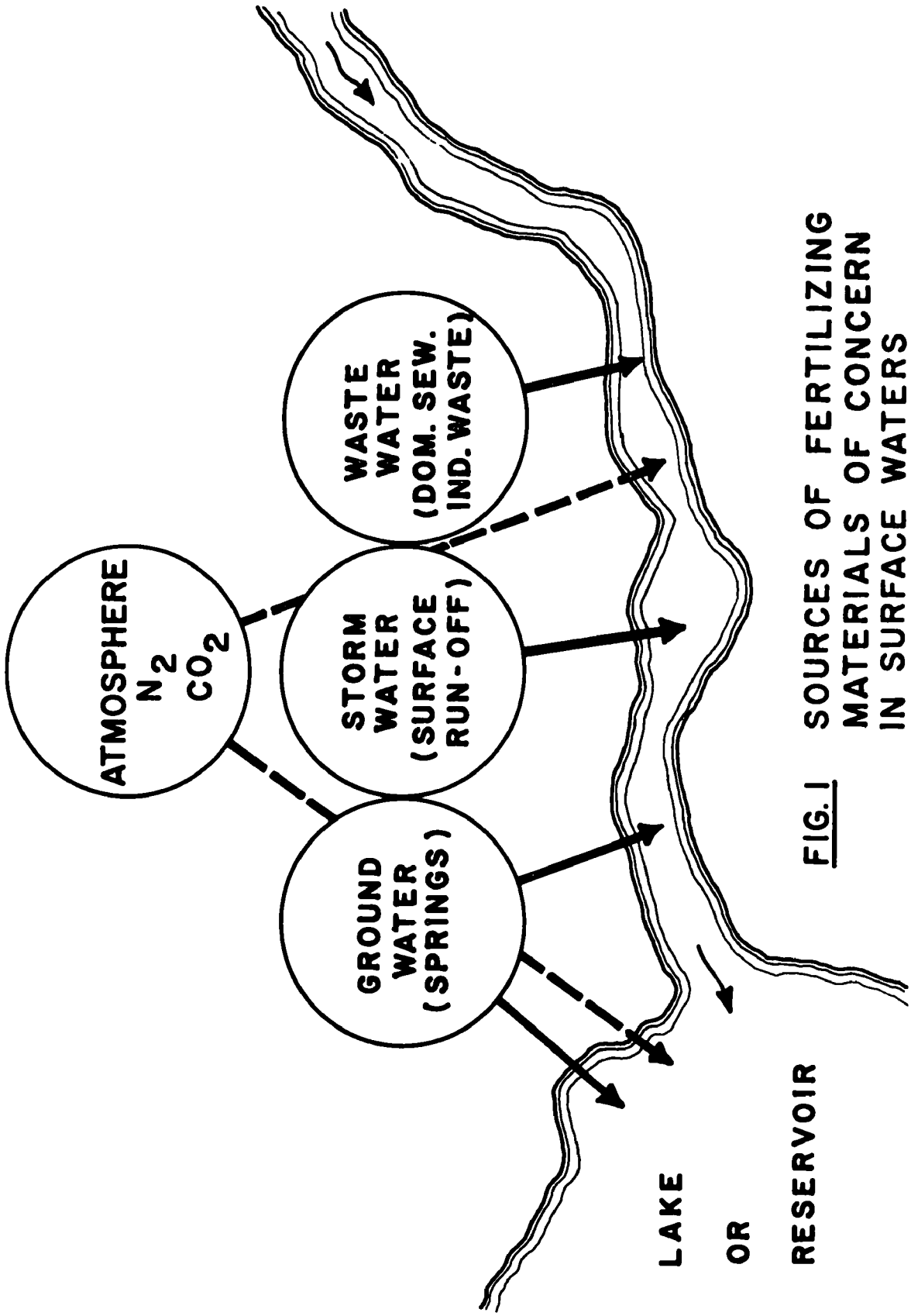

FIG. 1 SOURCES OF FERTILIZING MATERIALS OF CONCERN IN SURFACE WATERS

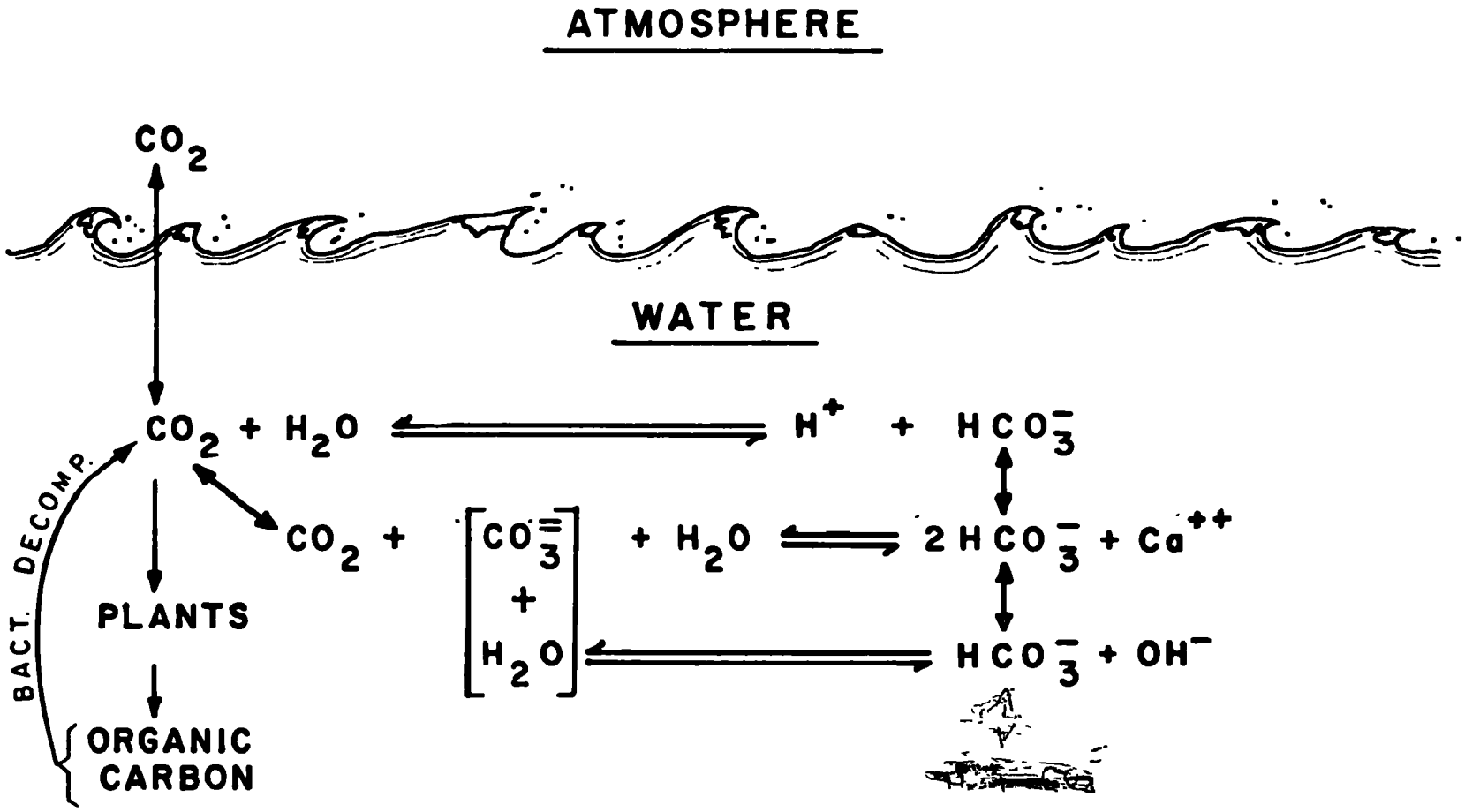

FIG. 2 CARBON DIOXIDE - BICARBONATE - CARBONATE - HYDROXIDE RELATIONSHIPS IN NATURAL WATERS

Also, may be unsuccessful in control unless phosphorus is controlled, too, because of nitrogen fixing forms.

2) Phosphorus removal - Phosphorus can be effectively removed by coagulation methods employing lime, alum or ferric salts. It is expensive and no one has proven its value beyond laboratory experiments.

d By Biological Engineering

Laboratory studies have shown that effluents essentially free of plant fertilizing elements can be produced by biological treatment of wastes with proper ratios of C to N and P.

3 Experiences

a Madison

b Detroit Lakes

c State College

d Lake Winnisquam, N.H.

E Practical Aspects

1 Diversion

2 Nutrient control

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CONTROL OF INTERFERENCE ORGANISMS IN WATER SUPPLIES

I NECESSITY FOR DATA

A Information on the number, kinds, and effects of interference organisms in a particular water supply is essential for determining adequate control measures.

B Collection of the biological data should be on a regular routine basis.

C Interpretation of data requires information on relationship of number and kinds of organisms to the effects produced.

D It is generally more satisfactory to anticipate and prevent problems due to these organisms than it is to cope with them later.

II CONTROL IN RAW WATER SUPPLY

A Use of algicides

1 Application of an algicide is to prevent or destroy excessive growths of algae which occur as blooms, mats or a high concentration of plankton.

2 Algicide may be applied to control even low concentrations of certain algae such as Synura.

3 Copper sulfate is the only algicide in common use at present.

a Application may be by dusting, spraying or dissolving from a porous container over all or part of the water surface, or by continuous feeding of the algicide at the intake of the reservoir or pre-treatment basin.

b Effective dosage depends upon the Alkalinity and pH and temperature of the water and the amount and kinds of algae to be controlled. Bartch states that the following arbitrary dosages have been found to be generally effective and safe:

M.O. alkalinity > 50 p.p.m. = 2 p.p.m. in the surface foot of water only (5.4 pounds per acre).

M.O. alkalinity < 50 p.p.m. = 0.3 p.p.m. in total volume of water (0.9 pound per acre foot).

c Application of copper sulfate should be limited to the minimum effective dosage because of its corrosive properties, and its toxicity to fish and other aquatic animals.

4 Other algicides

a Promising types include inorganic salts, organic salts, rosin amines, antibiotics, quinones, substituted hydrocarbons, quaternary ammonium compounds, amide derivatives and phenols. Cuprichloramine which is a combination of copper, chlorine and ammonia, and also chlorine dioxide have shown promise as general algicides.

b For domestic water supplies they will have to be not only economically feasible but nontoxic to animal life and to green plants other than algae.

c Due to higher costs they will probably be used only when adequate plankton and algal records are kept, which would permit early localized treatment.

d Algicides selectively toxic to the particular algae of greatest significance would be useful.

5 Mechanical removal or spreading out to permit rapid drying may be the simplest way of handling massive growths which are detached and washed ashore.

6 Turbidity due to silt keeps down the plankton population. In shallow reservoirs, fish which stir up the bottom mud will aid in keeping turbidity due to silt high.

7 Provisions for keeping the amounts of nutrients to a minimum may be emphasized more in the future.

8 For new reservoirs, clearing the site

of vegetation and organic debris before filling will reduce the algal nutrients. Steep rather than gentle slopes will reduce the areas which allow marginal growths to occur.

III CONTROL IN TREATMENT PLANT

A Coagulation and sedimentation

1 When well regulated they often will remove 90 per cent or more of the plankton.

2 With low plankton counts, a coagulant aid may be required.

3 Frequent removal of sludge from the basins, especially during the warm seasons may help to reduce tastes and odors originating from decomposing organic sediment.

B Sand filtration

1 Both slow and rapid sand filters tend to reduce the plankton count of the effluent by 90 per cent or more, when well regulated.

2 For rapid filters, accumulated plankton can be removed or reduced by surface scraping and by back washing.

C Micro-straining

1 This involves the passing of the water through a finely woven fabric of stainless steel. All but the smaller plankton organisms tend to be removed from the water. It is being used in some treatment plants in England and elsewhere.

D Activated carbon

1 The slightly soluble, organic, taste and odor compounds tend to be readily adsorbed by the activated carbon. It is probably most often applied prior to coagulation, but may be used prior to filtration or in the raw water.

E Chlorination

1 Treatment with chlorine is practiced primarily to destroy pathogenic organisms. The dosages commonly used are toxic also to many algae and to some of the other groups of aquatic organisms. However, dead as well as living organisms are often capable of causing tastes and odors and of clogging filters.

F The depth and position of the intake for entrance of raw water into the treatment plant may determine the kinds and amount of plankton which will be drawn into the plant. Plankton algae generally are more concentrated near the surface of the water in lakes and reservoirs.

IV CONTROL IN DISTRIBUTION SYSTEM

A Maintenance of a chlorine residual controls the chlorine sensitive organisms.

B Other pesticides such as cuprichloramine have been used in attempts to control the resistant organisms such as worms, nematodes and copepod eggs.

C Flushing of infested portions of the system, especially dead ends may be practiced.

D Covering of treated water reservoirs to prevent the entrance of light will stop the growth of algae.

E Organisms associated with pipe corrosion are probably the most active when the water itself is corrosive.

F Mechanical cleaning of the distribution system may be an effective but expensive method of reducing infestations of attached organisms.

V SUMMARY

A Adequate control is dependent upon adequate procedures for detecting and recording of organisms.

B Control may involve the following·

1 Use of an algicide or pesticide.

2 Mechanical cleaning of distribution lines, settling basins, and filters, screens, intake channels and reservoir margins.

3 Modification of coagulation, filtration, chemical treatment or location of raw water intake.

4 Use of adsorbent, such as activated carbon, for taste and odor substances.

5 Modification of reservoir to reduce the opportunities for massive growths.

a By covering treated water reservoirs

b By increasing the depth of the water

c By eliminating shallow marginal areas

d By reducing the amount of fertilizing nutrients entering the reservoir

e By encouraging a balanced development of the aquatic organisms

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NUTRIENTS: THE BASIS OF PRODUCTIVITY

I INTRODUCTION

A Nutrients of importance include macronutrients: those needed in large quantities, and micronutrients: those needed in small amounts.

B These nutrients are important because they promote biological responses which may interfere with some desired use of the water by man.

C Other factors (e.g. temperature, light) affect the use of these nutrients and should be considered in an evaluation of the effects of nutrients upon the ecosystem.

II Algae, bacteria, fungi and aquatic plants are the forms of life which nutrients affect most directly.

A Algae are of Several Types

1 Phytoplankton are small algae suspended in the water and form the basis of productivity in the aquatic environment.

2 Benthic algae are those forms anchored to substrates of rock and bottom materials.

B Aquatic plants are of several types. In general they may be referred to as rooted or floating forms.

C Heterotrophic bacteria are fungi which respond to organic nutrients introduced into water. Autotrophic bacteria may respond and grow due to inorganic nutrient sources.

III BIOLOGICAL LAWS

A Liebig's "law" of the minimum: the essential material available in amounts most closely approaching the critical minimum needed will tend to be the limiting.

B Shelford's "law" of tolerance: survival of an organism can be controlled by the quantitative or qualitative deficiency or excess with respect to any one of several factors which may approach the limits of tolerance for that organism.

C Q_{10} "law": with a temperature increase of 10 degrees centigrade metabolic processes (rates) are approximately doubled.

IV The process of photosynthesis is the fixation of the sun's energy with the production of organic matter by plants.

A The general reaction is given below:

$$CO_2 + H_2O \xrightarrow{\lambda} CH_2O + O_2$$

B Chlorophyll contains basically C, O, H, N and Mg, and in general makes up about 5% of the dry weight of algal cells.

V MEASUREMENT OF PHOTOSYNTHESIS

A Oxygen production can be used as a measure of photosynthesis because for each mole of CO_2 reduced to organic carbon one mole of free oxygen is liberated.

1 The value of O_2/CO_2 has been found experimentally to be 1.25 rather than 1.0.

B CO_2 Assimilation

1 The CO_2 taken up by algae does not all originate from the dissolved gas. Some algae can use bicarbonate directly as a source of carbon.

2 Hence measurement of CO_2 uptake from water is a complicated problem which must consider pH, HCO_3^-, and $CO_3^=$ concentrations.

C Fixation of Carbon-14

1 The use of C^{14} as a tracer of C^{12} in plant metabolism and productivity estimation has been widely used since the early nineteen fifties.

2 In this method a known amount of C^{14} is added to the water and after a period of time the proportion of C^{14} in the plant cells to C^{14} added is found. The amount of carbon assimilated is then estimated from the following equation.

$$\frac{\text{activity of phytoplankton}}{\text{activity of } C^{14}O_3^= \text{ added}} (K) = \frac{\text{total carbon assimilated}}{\text{total carbon available}}$$

3 Where K is a constant relating to the slower uptake of C^{14}.

4 The total carbon available is determined chemically.

D Uptake of Mineral Nutrients

1 The measurement of depletion of nutrients in solution has been tried but found unreliable.

E Chlorophyll

1 The quantity of chlorophyll present has been found to bear some relation to productivity but not a reliable one.

VI Nutrients of significance in the growth and production of algae and plants are discussed below.

A Carbon

1 Sources

a Gaseous CO_2

b HCO_3^-

c $CO_3^=$

d Other carbon compounds

2 Effects of the removal of carbon upon the water

a Lowered pH

b Deposition of $CaCO_3$

3 The quantity of carbon available is great and it usually is not a limiting factor.

B Nitrogen

1 Nitrogen can be taken up by most algae as either ammonium salts or as nitrates. Nitrites can also be used but a high concentration is usually inhibitory. Some blue green algae can fix atmospheric nitrogen. Certain algae varieties require supplementary amines, growth factors, etc.

2 The quantity of nitrogen in waters has definitely been shown to limit algal populations.

C Phosphorus

1 Phosphate seems to be the only inorganic source of this nutrient.

2 Limiting concentrations of P have been found to range from .05 ppm at a minimum and an inhibitory affect if P concentrations exceed 20 ppm.

3 Optimum concentrations have been found to range from .018 ppm to 15 ppm.

4 Storage of inorganic phosphate by algae has been demonstrated. The extent of this storage may reach 80% of the total phosphorus in algal cells.

D Silicon

1 Nutrient ratios in the algal cells of some areas have been found to be Si 23: N 16: P 1. It can be seen from this ratio that silicon is an important element in algal growth.

2 Silicon is especially important in the population growth of diatoms and may be the limiting growth factor in these populations.

E Inorganic micronutrients - Many elements are needed in very small quantities by algal cells. Some of these have a known function in algal metabolism; others do not.

1 Mg is a cation of major importance in the chlorophyll molecule.

2 Co is known to be necessary for vitamin B_{12}.

3 Mn is necessary for several enzyme systems.

4 Mo, V, Zn, and Cu are necessary but these functions are not as well known.

F Organic Micronutrients

1 Of 179 algal strains investigated about 40% required vitamin supplementation for optimum growth. Principal growth factors that were not synthesized in sufficient quantity are given as follows along with the percentage of the vitamin deficient strains showing marked productivity gain after supplementation:

a B_{12} addition increased growth on 80% of the strains.

b Thiamin addition increased growth on 53% of the strains.

c Biotin addition increased growth on 10% of the strains.

2 Algae can use and may require many organic compounds depending upon environmental conditions and the ability of the organism to synthesize required building blocks from mineral forms of C.N. & P. This is an area for continued investigation with many unappreciated or vaguely understood ecological factors.

VII PROBLEMS AND BENEFITS RESULTING FROM ALGAE PRODUCTION

A Problems to man may result when the total "primary production" by algae leads to an increase in the total organic content of the water that interferes with a desired use.

1 This may consist of a high algal population that produces a water with high turbidity, taste and odor, or other undesirable effect. High respiratory needs may lead to nocturnal oxygen deficit.

2 Certain algae may cause tastes and odors, clog filters; or otherwise interfere with potable water processing.

3 Death of large algal populations may lead to tastes and/or odors through bacterial decomposition. Oxygen deficits may result at any time of day in this process. Deposition of masses of organic sediment or sludge may be considerable.

4 Other problems might be cited.

B The primary production of algae can also serve as a supply of food to consumer organisms (animals), resulting in increased production at several (trophic) levels:

zoömicrobes, microinvertebrates, macroinvertebrates, fishes.

1 Earlier notation cited the release of oxygen during utilization of CO_2 during algal photosynthesis. This encourages fungal or bacterial breakdown of pollutants.

2 Photosynthesis occurs in the presence of adequate light and favorable conditions. In darkness, the cells continue to respire and may consume more oxygen than they produced because photosynthesis increases the organic load.

3 Photosynthesis tends to occur at the surface where light intensity is greatest. Poor vertical mixing would result in stratification of water supersaturated with oxygen over oxygen deficient water. Depending upon conditions, a significant fraction of the oxygen could be lost to the atmosphere.

4 Increased productivity may result in temporary reduction of the free dissolved nutrient level in the water but harvesting at some level is essential to prevent later recycle.

VIII CYCLE OF NUTRIENTS

A Once nutrients enter a body of water they are cycled through a food chain.

B Factors affecting this food chain (e.g. toxicity, removal) will affect the concentration and distribution of the nutrients.

ACKNOWLEDGEMENT:

This outline contains certain material submitted by F.J. Ludzack and H.W. Jackson.

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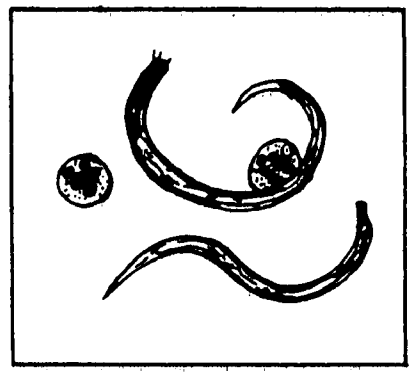
This outline was prepared by Michael E. Bender, Biologist, Formerly with Training Activities, Ohio Basin Region, SEC.



CHAPTER VI

RELATED STUDIES

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THE PROBLEM OF SYNTHETIC ORGANIC WASTES

I Sources of organic chemicals in water are varied and of differing complexity.[1]

A Natural pollutants, such as algae, actinomycetes, etc. contribute to organic pollution.

1 Tastes and odors associated with these materials are probably not merely a result of decomposition, but are closely associated with materials produced during the life cycle of the organisms and plants.

2 Discharge of nutrients in the form of phosphorus and nitrogen compounds from domestic or other wastes frequently stimulate the production of natural pollutants.

B Industrial wastes, due to the rate of population increase and industrial expansion, have made the problem of effective water treatment an acute one in many places.

1 The production of synthetic organic chemicals has risen steadily over the past years, representing many new and complex products - and of importance to us - new and complex wastes.

2 The ideal method of handling industrial waste is at its source.

a However, what is often considered good treatment, still results in materials present in sufficient quantities to affect the taste and odor of water.

b Many problems are caused by slug discharges, often accidental.

C Domestic wastes in various stages of treatment.

D Miscellaneous sources also contribute to the problem.

1 Wastes from private and commercial boats.

2 Chemicals applied to the land may be washed into streams.

3 Chemicals applied directly to water.

a Evaporation control

b Killing off rough fish

c Aquatic plant control

II Concentrations of organic chemicals in water, even in comparatively minor quantities may cause difficulties

A Wastes may contain from a few mg/l to several hundred mg/l of organic contaminants.

B Surface waters may contain from a few µg/l of organics to several mg/l.

1 Some of the chemicals isolated from water, along with the concentrations which can be detected by odor, are:[2]

Substance	Concentration Detectable*, µg/l
Formaldehyde	50,000
Picolines	500 - 1,000
Phenolics	250 - 4,000
Xylenes	300 - 1,000
Refinery hydrocarbons	25 - 50
Petrochemical waste	15 - 100
Phenyl ether	13
Chlorinated phenolics	1 - 100

*Concentrations were determined by taking the median of 4-12 observations.

III The damaging effects of organics in water are becoming more apparent.

A Taste and odor in water is usually the first noticed effect from organics. This is a serious public relations and economic problem; it also may be a health problem.

B Organic contaminants may interfere with coagulation, damage ion exchangers, and create chlorine and carbon demand.

C In the stream they may have adverse effects on aquatic forms that support higher aquatic life, cause off-flavors in fish flesh, or have direct toxic effects on fish.

IV The methods of study employed in the collection and identification of organic chemicals in water involve physical and chemical methods and instrumental analysis.

A The comparatively small amounts of organic materials may be concentrated by adsorption on activated carbon.

1 This carbon is then extracted with appropriate organic solvents, the solvent extract is taken to dryness, the weighed extract is subjected to solubility group separation, and these individual groups may then be analyzed by various methods.

2 Employing the above method on Ohio River water, the following results were obtained:[3]

Chemical Group	% of Total	TOC in µg/l	Relative Odor Contribution
Water solubles	20	860	23
Ether and water insolubles	22	110	200
Neutral	14	3	4,670
Amine	4	575	7
Weak acid	8	645	12
Strong acid	6	365	16
Amphoteric	10	5,000+	--
Loss	16	--	--

B Chemical separation and analyses may be accomplished by means of column chromatography, formation by derivatives, gas chromatography, infrared and ultraviolet spectroscopy, x-ray diffraction, etc.

1 Specific organic chemicals recovered from river and drinking waters by these methods include: synthetic detergents (ABS), phenylether, phenol, DDT, aldrin, o-nitrochlorobenzene, α-conedendrin, and xylene.

V Some of the types of problems that may be attributed to organic wastes, and more specifically to problems of taste and odor, may be represented by the following examples:

A By applying the previously mentioned carbon adsorption method, the odor potential of organic pollutants and the dilution necessary to reduce this odor potential to a barely perceptible level has been determined:[4]

Industry Source	Conc. Required for Detectable Odor µg/l		Dilution Factor	
	$CHCl_3$ Sol. Org.	Total Org.	$CHCl_3$ Sol. Org.	Total Org.
Brewery	770	1,400	14	86
Chemical	28	32	11,000	14,000
Corn Refining	1,000	3,600	1.4	2.1
Meat Packing	1,200	3,600	92	140
Metal Fabrication	890	1,600	2.8	4.8
Paint	390	1,000	69	98
Pharmaceutical	290	340	10	32
Refinery	84	510	780	760
Soap	900	1,800	640	350

B Of all the organic pollutants that can affect the taste and odor of drinking water, phenol has been the most extensively studied.

1 The potential sources of this chemical, both natural and synthetic, have been discussed.[5]

2 The course of chlorination of phenol, a common method of treatment in the water plant, has been shown to proceed by a process which starts with the pure compound (in itself relatively tasteless) and proceeds through strong-tasting intermediates to tasteless end products.[6]

C The effects of petrochemical wastes[7] on water quality are becoming increasingly important, it has been predicted that by 1970 the petrochemical production on a tonnage basis may be equal to 41% of all chemicals.

1 The three principal groups of petrochemicals are the paraffins, the naphthenes, and the aromatics. From these, over 200 basic products are manufactured, having thousands of subordinate uses.

2 Correspondingly, more than 100 identifiable compounds have been found in waste streams from petrochemical processes.

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This outline was prepared by R.L. Booth, Chief, Analyses Unit, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, OH 45202.

BEHAVIOR OF RADIONUCLIDES IN FOOD CHAINS
FRESH WATER STUDIES

INTRODUCTION

Fresh water lakes and streams, in their traditional roll of receiving and transporting all manner of materials, seem destined to continue this function in the atomic age and have radionuclides. In many cases the water may serve as an effective medium through which the nuclides can become incorporated into living organisms. Ultimately some of the radioactive materials may be deposited in fish or crops which are eaten by man and thus provide an additional source of radiation exposure.

This discussion is primarily directed toward the mechanisms through which aquatic forms may accumulate radioactive materials from their environment and the manner in which we may expect certain nuclides to become distributed in biological systems of fresh water lakes and streams. In a broad sense, the subject of the behavior of radionuclides in fresh water food chains might also include irrigated crops, and animals which drink from streams and ponds. Such problems, are however, more closely allied with terrestial studies and in this case consideration will be given only to those forms which actually live in the water.

SOURCES

Fresh water streams may receive radioactive materials from a great variety of sources. Naturally occurring radioisotopes removed from the atmosphere and eroded from radioactive ores have been carried in both surface and underground waters since early in the history of the earth. Typically, U^{238}, Ra^{226}, and Th^{232} are present in measurable amounts, but other isotopes such as K^{40} and C^{14} are also present. The background levels are in the range of 10^{-9} to 10^{-10} μc/ml for most surface waters [1] but values in excess of 10^{-4} μc/ml have been reported for certain hot springs of high radium content. In most streams the natural concentration of the heavy metals is so low and their uptake by aquatic forms is so small that they contribute but little to the radioactive content of the organisms. Most of the natural radioactivity in the biota originates from K^{40} which, in fish, amounts to about 3×10^{-6} μc/g.

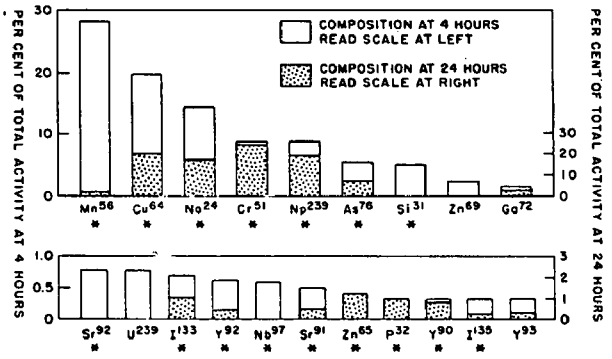
With the creation of a demand for large amounts of uranium as a basic source of nuclear energy the uranium ore refining industry has expanded rapidly during the past decade. As pointed out by Tsivoglou, et al. [2], the concentrations of radioelements of the uranium-radium family are substantially increased in streams which receive the effluent directly from uranium mills or seepage from ponds used to retain their tailings. The concentration of radium in the biota of the streams is likewise increased.

Liquid wastes from the fabrication of refined uranium into fuel for reactors can be another source of naturally radioactive isotopes to streams. In comparison with releases at the mills and with the problems which accompany irradiation of the fuel, however, the contribution from the fabrication plants will usually be quite insignificant.

The large quantities of artifically radioactive nuclides produced in reactors form a new type of disposal problem, the handling of which requires a technology considerably more complex than that associated with the natural isotopes. Dependent upon individual design, the reactors themselves may or may not contribute significant quantities of radionuclides to fresh water streams. The megacurie quantities of fission products which accumulate in the reactor fuel do not, under ordinary circumstances, escape from the fuel while it is in the reactor. As pointed out by Terrill [3], "Present practice involves extensive control measures through design and operation to confine the fission products within the reactor core and its immediate environs." Failure of the cladding of fuel elements does, of course, provide the opportunity for entry of fission products into the primary coolant stream or moderator. Under normal circumstances, the principal source of radioactive materials in the fluids which pass through the reactor core is the activation of materials which are exposed to the neutron flux. These activation products may originate from materials dissolved in the coolant or they may corrode from

the surfaces of the fuel elements, cooling system, or structural materials. In the Hanford-type reactor, the coolant is filtered Columbia River water which is returned to the river after a single pass through the reactor. Figure 1 shows the isotopes which predominate in effluent from the Hanford reactors four hours after irradiation. Many of these have such short half-lives that they have decayed to very low levels by the end of the first day. Minor amounts of fission products are also found in the Hanford reactor effluent not only as a consequence of the occasional failure of a fuel element, but also because of natural amounts of fissionable uranium in the Columbia River water. The release of radioactive effluent to the Columbia River is accompanied by extensive monitoring, both before and after discharge, in order to provide assurance that the radiation exposures to people who subsequently use the river or eat fish or irrigated crops are well within permissible limits.

Figure 1



RADIOELEMENT CONTENT OF REACTOR EFFLUENT 4 HOURS AND 24 HOURS AFTER IRRADIATION. (Radioelements marked * ore are routinely measured).

"Radioactive Waste Management Operations at the Hanford Works", included in Congressional Hearings on Industrial Radioactive Waste Disposal (1959).

Thd kinds and amounts of radionuclides discharged to the Columbia River with the Hanford reactor effluent should not be considered as characteristic of other types of reactors. The concentrations of various activation products which build up in the primary circulating fluid will be dependent upon the intended chemical composition of the fluid, impurities present, the corrosion of cladding on fuel elements, piping and vessels, film formation, neutron energy and flux, radioactive half-life, and the effectiveness of ion-exchange "clean-up" loops where these are used. Significant amounts of activation products of common elements ranging from tritium to neptunium can conceivably be present. In most cases there probably will not be intentional release of radioactive materials accumulated in the re-circulated fluids directly to fresh water streams. However, minor leakage from the complex piping and fuel handling systems into the plant effluents appears almost inevitable.

Excluding nuclear detonations, the largest potential source of radionuclides to the environment is the fission products which accumulate in reactor fuels. At the present time, the bulk of the high-level waste which results from the processing of the fuel elements, is confined in some artificial or natural container. Even so, large quantities of water with low or intermediate concentrations of radionuclides result from the later stages of most separation processes and these must usually be disposed of at, or very near, the processing plant. In many cases, disposal of such wastes will be to the ground or to pits on the surface in order to take advantage of the ion-exchange capacity of the soil and the time lags which allow for decay of the isotopes with short and intermediate half-lives. At the Hanford plant the cumulative volume of intermediate-level waste discharged to the ground through 1958 amounted to nearly 4 billion gallons, containing nearly 2 million curies of beta-emitters. The topography, soil structure, and geology at Hanford favor retention and none of this material has been detected in the Columbia River[4]. Conditions for surface disposal are less favorable at other existing major installations, and wastes containing fission products discharged to pits at both Oak Ridge[5] and Chalk River[6] have seeped through the soil and appeared in streams and lakes which receive the drainage.

Radioactive wastes may also enter fresh water streams via the drains from industrial, medical,

or research facilities which use isotopes. Although the amounts involved are comparatively small, such sources should not be entirely disregarded since the Oak Ridge National Laboratory is now shipping on the order of 150,000 curies of isotopes a year to domestic users.

On the basis of high yield from nuclear fission, significantly long half-life, and biological importance, the fission products which one would expect to find in greatest abundance in the biota of fresh water streams and lakes are: Sr^{89}, Sr^{90}, Y^{91}, Zr^{95}+ Nb^{95}, Ru^{106}, I^{131}, Cs^{137}, Ba^{140}, Ce^{144}, and one or more rare earths. In addition to these, neutron activation products which appear to be of importance include: P^{32}, Sc^{46}, Cr^{51}, Mn^{54}, Fe^{55}, Fe^{59}, Co^{57}, Co^{60} and Zn^{65}. In the immediate proximity to reactors, very short-lived isotopes such as Na^{24}, Cu^{64}, and As^{76} may be detected.

As a result of the use of nuclear energy, the associated mining and refinement of fissionable materials, and the widespread use of radioisotopes, the radioactivity in a few surface waters of the world has now been increased above the original background level. As the use of atomic energy and its byproducts becomes more extensive, the numbers of rivers and lakes which receive radionuclides will increase, and the organisms which inhabit these waters will also acquire the radioactive materials. Some appreciation of the mechanisms involved in the uptake of radionuclides by aquatic organisms is necessary to anticipate potential sources of exposure to man, and, if necessary, to effectively control or reduce the amounts accumulated.

UPTAKE BY LIVING ORGANISMS

Radioactive materials present in the water may be taken up by aquatic organisms via three different mechanisms: 1. adsorption onto exposed surface areas, 2. absorption through membranes exposed to the surrounding water, possibly with the aid of active transport mechanisms, and 3. engulfment of food or inert particles which contain the nuclides. The relative importance of these mechanisms is different for various kinds of organisms and even similar species. It also fluctuates widely between different elements and different environments.

Adsorption, which is a physical process, occurs very rapidly. It is of greatest significance among those organisms which have large surface to volume ratios such as sponge and diatoms. A clear distinction between adsorption and biological absorption is not easy to make however, since the physical process of adsorption may be a necessary primary step in the movement of ions into the cell. Films of bacteria and other microorganisms may also fix radioactive materials on the surfaces of organisms to give an adsorption-like effect.

The process of absorption is obviously of prime importance to the plants since this is the means by which nutrient materials are removed from solution and metabolized. Many non-essential elements will also enter the plant cells because of passive diffusion through the membranes or because the organism is handling them in a manner similar to the essential elements. The uptake of radioactive materials by the plants is, of paramount importance to the higher forms since the plants form the food base.

Direct absorption of some radioisotopes from the water also occurs in aquatic animals, including the fish, with the gill membranes probably playing the important role. Such direct uptake by fresh water fish has been demonstrated for Sr^{90}(7) (8) (9), Ba^{140} + La^{140} (7), Ca^{45} (10) (11) (12), P^{32} (13) Na^{24} (7), and Cs^{137} (14). However, the dominant means by which many radioactive materials are accumulated by aquatic animals is through ingestion, since this is the manner in which they obtain the bulk of their nutrient materials. Ingestion does not insure that the isotope will be assimilated through the gut. Some materials may pass through the gut with virtually no uptake, and seldom, if ever, will all of the ingested radioactive material be metabolized. For example, Watson[15] found that after 24 hours, rainbow trout retained about 60 per cent of the radiophosphorus which they ingested. Schiffman[16] found that only about 7 per cent of Sr^{90} incorporated into natural food organisms was retained by trout after one day.

CONCENTRATION FACTORS

The uptake of radionuclides by organisms is obviously not a phenomenon which is peculiar to the radioactive forms of the elements. Rather, the radioisotope acts only as a tracer to demonstrate the difference between the abundance of the element in the water and in the organism. The so-called "concentration factor"

$$\frac{(\mu c/g \text{ of organism})}{(\mu c/ml \text{ of water})}$$

for any radionuclide cannot exceed the ratio which exists between the concentrations of the element in the organism and in the water. On this basis it should be possible to predict the ultimate concentration factors for various radionuclides from conventional quantitative chemical measurements, provided the nuclide has become distributed throughout the system and is uniformly mixed with its stable counterpart. In practice, however, it may be quite difficult to measure the concentration of some elements because of the minute quantities involved.

Estimation of concentration factors for fresh water organisms involves, at the present time, a considerable amount of speculation. While the concentration of some elements in a variety of marine organisms is available,(17) and the chemical composition of sea water is relatively stable, there is very little information readily available on the chemical composition of fresh water organisms. Chemistry of various lakes and streams differs so greatly from one region to another that no generalizations can be made. The picture is clouded further by the fact that the concentration of many elements in aquatic forms is partially dependent upon their abundance in the surrounding water. Although it is virtually impossible to write down probable concentration factors which will apply to more than one locality, an indication of the differences in the orders of magnitude of the concentration for a few of the common elements in some organisms and in water is shown in Table 1.

It is evident then, that radioisotopes of the different elements will be concentrated to various amounts by the different organisms. The concentration of phosphorus in Columbia River water is about 0.01 ppm, while in the fish it amounts to about 6,000 ppm. These values would lead to an estimated concentration factor of about 600,000 for the fish. Since radiophosphorus is one of the isotopes present in the effluent which is discharged into the Columbia River from the Hanford reactors, the actual concentration factor for this nuclide can be measured. In contrast to the theoretical value of 600,000, the maximum observed value is only about 100,000. The observed value is less because the biological processes required to deposit and exchange the phosphorus in the body of the fish are long in comparison to the radioactive half-life of P^{32}, which is about 14 days. Consequently, the radioisotope is not uniformly mixed with all of the stable phosphorus involved in the system. Such a reduction would not be expected for long-lived isotopes.

Table 1

Concentration (ug/g wet weight or ml) of some elements in fresh water organisms and in some major rivers of the United States

Element	Organism 1/			Water 2/	
	Algae (Spirogyra)	Insect Larvae (Caddis Fly)	Fish (Minnows)	Low	High
Silicon	1,500	20	10	3	20
Sodium	1,500	700	1000	1	200
Phosphorus	250	400	6000	<0.001	1.5
Calcium	1,500	300	3000	2	200
Iron	6,500	300	1	<0.01	6.0
Strontium	2	0.2	0.3	0.005	1.9

1/ Values are only estimates of orders of magnitude obtained by spectrophotometric analysis at the Hanford Laboratories. They are recorded here to illustrate differences which can exist and are not intended for use in precision work.

2/ Abstracted from Moyle, J. B., "Relationships between the chemistry of Minnesota Surface Waters and Wildlife Management". Journal of Wildlife Management 20: 303-320 1956, and Clarke, F. W., "The Composition of the River and Lake Waters of the United States". U. S. Geological Survey, Prof. Paper 135, 1924. Strontium values from Alexander, Nusbaum, and MacDonald, "Strontium and Calcium in Municipal Water Supplies", A. W. W. A., Journal 46(7): 643-654, (1954).

From measured amounts of radionuclides in the effluent from Hanford reactors and in organisms living in the Columbia River immediately below the reactors, concentration factors have been calculated for the more abundnat isotopes. These are listed in Table 2 for the season when maximum values occur.

The concentration factors for As^{76}, Sc^{46}, Cr^{51}, and Cu^{64} are quite low in fish in comparison to those in algae and insect larvae. This illustrates that food chains, which on the one hand provide the mechanism for transferring radioactive materials from one organism to another, can also serve, in some cases, to reduce the concentration of radionuclides in large animals. Two different processes can be involved. Where possible the organisms will eliminate non-essential elements, and thus there will be a repeated selection against such nuclides along the food chain; and short-lived isotopes will decay to low levels as they pass through the chain.

Table 2*

Observed Concentration Factors $\left(\frac{\mu c/g \text{ of organism}}{\mu c/ml \text{ of water}}\right)$

For Significant Isotopes Found in Columbia River Organisms

Isotope	Algae[a]	Insect Larvae[b]	Fish[c]
P^{32}	100,000-1,000,000	100,000	100,000
Zn^{65}	100,000	10,000	1,000-10,000
Cs^{137}[d]	1,000-5,000	1,000	5,000-10,000
Sr^{90}	10,000	100	1,000
Na^{24}	100	100	100-1,000
As^{76}	10,000	1,000	100
Sc^{46}	100,000	1,000	10
Cr^{51}	100-1,000	100-1,000	10
Cu^{64}	10,000	1,000	10

Many of these values are not yet well defined and are subject to revision.

[a] Stigeoclonium sp. [b] Hydropsyche sp. [c] Richardsonium sp.

[d] Data for Cs^{137} obtained from a pond environment rather than the Columbia River and species are different from those listed above.

(Values are based partly on data reported by J.D. Davis, R.W. Perkins, R.F. Palmer, W.C. Hanson, and J.F. Cline (18) and R.C. Pendleton and W.C. Hanson (19).)

*This table reproduced from "The Need for Biological Monitoring of Radioactive Waste Streams," by R.F. Foster presented at the Nuclear Engineering and Science Conference April 6-9, 1959, Cleveland, Ohio.

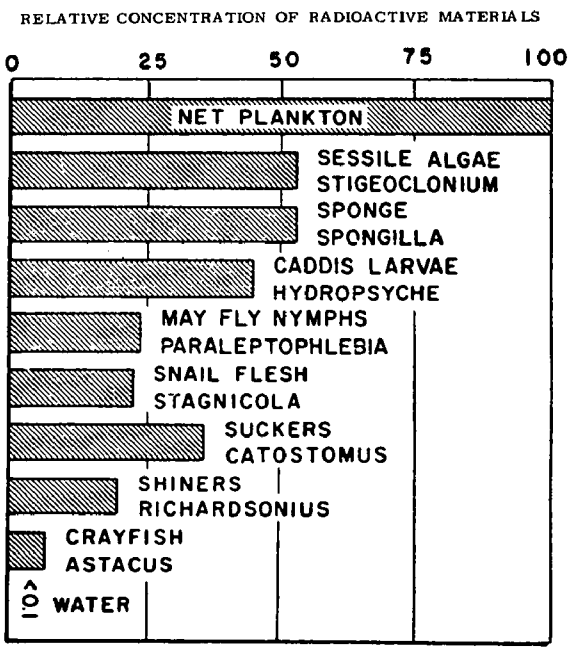
The isotopes which are most likely to have high concentration factors in fish and other large aquatic animals are those which are readily assimilated, which are retained for relatively long periods of time in various body structures, and which have long radioactive half-lives. These are characteristics which result in relatively high body burdens for humans as well, and which influence a low maximum permissible concentration for the isotope, in drinking water or food.

DIFFERENCES BETWEEN SPECIES

Differences in the concentration factors for various isotopes among different species leads, to different levels of radioactivity in the organisms. Columbia River organisms provide a good example of this as shown in Figure 2. The short-lived isotopes contribute significantly to the activity density (quantity of radioactive materials per unit mass of substance) of plankton, algae, and sponge. But in the higher animal forms, about 95 per cent of the activity originates from P^{32}.

Figure 2

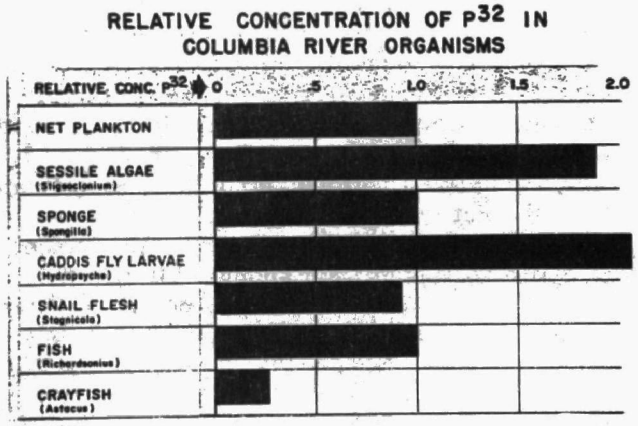
Radioactivity in Different Columbia River Organisms



Davis and Foster, "Bioaccumulation of Radioisotopes through Aquatic Food Chains", Ecology 39: 530-535 (1958)

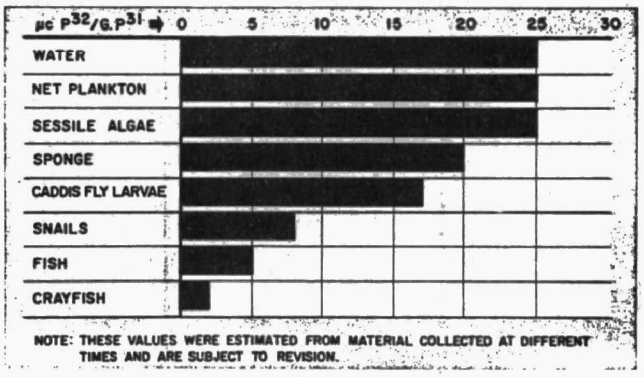
Because of the dominance of the P^{32}, it is of interest to consider this isotope separately from the others. Figure 3 shows the relative concentrations of P^{32} in most of the species included in Figure 2. The different concentrations result from a combination of several factors: the chemical composition of the various species is different, the moisture and organic content varies, and there are different lag times along the food chain which permit radioactive decay.

Figure 3



As mentioned before, the reduction in the concentration of a radioisotope of an essential element along the food chain may occur with relatively short-lived isotopes because the ratio of radioactive to non-radioactive atoms of the element does not remain constant throughout the entire biological system. This ratio may properly be called the specific activity since it is the concentration of the isotope per gram of stable element - in this case - μc P^{32} per gram of P^{31}. The Columbia River below the Hanford reactors provides an unusual opportunity for the study of the specific activity of P^{32} in various organisms of the food chain, since a near steady state is maintained. A "fresh" supply of the isotope is added to the river water at a more or less constant rate with the reactor effluent. Since the concentration of stable phosphorus in the river water is also relatively constant, the specific activity of P^{32}, remains about the same in the water throughout much of the year. If there were a near instantaneous and complete exchange of phosphorus between the river water and all aquatic organisms, then the specific activity of the organisms would be identical with that of the water. Figure 4 shows that this is not the case. For plankton and sessile algae the specific activity is virtually the same as in the water: This indicates a very rapid exchange of phosphorus between these forms and the water. The specific activity is progressively less in the insect larvae, snails, fish, and crayfish (scavengers), indicating that replacement of phosphorus in these animals goes on at a relatively slow rate, and thus the phosphorus "ages" in the organism.

Figure 4



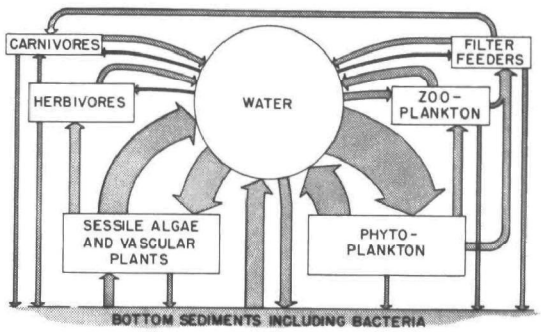
If one compares the specific activity of caddis fly larvae with that of their food, which is predominantly plankton for the species represented here, it can be shown that the average phosphorus atom remains in the caddis larvae for about 9 days.

The low specific activities of P^{32} in the larger animals results not only from slow phosphorus exchange within the animals, but also from the reduced specific activity of its food. The effect is thus cumulative along the food chain. For isotopes which are shorter lived than P^{32}, the reduction in specific activity along the food chain may be even more spectacular; for isotopes with relatively long half-lives the reduction may not be apparent. It must be remembered, however, that a reduction in specific activity along the food chain results from a combination of two factors: one is a comparatively short half-life, and the other is a relatively long retention of the element in the organisms.

EXCHANGE BETWEEN ORGANISMS

Although we talk in terms of food chains with the implication that radionuclides and other materials are transferred sequentially from small food species to larger predatory forms, such simple, straight-forward systems are not strictly characteristic of aquatic communities. Such communities might better be thought of as complex chemical exchange systems in which there is a kind of equilibrium established between the concentration of the nuclide in the water and in the various biological forms, silt, and other exposed surfaces. Several experiments have been carried out in both the laboratory and the field with the use of P^{32} in order to measure the rates of exchange between various components of the systems (20-31). Figure 5 is a highly schematic representation of the kind of phosphorus exchange which operates in a fresh water community. The size of the arrows is intended to give some conception of the quantity of phosphorus (or radiophosphorus in this case) moving in a given direction in some short unit of time. A large fraction of the available isotope moves rapidly back and forth between the water and the photosynthetic plants. Animals which eat the plants obtain an appreciable part of their radiophosphorus from the plants, but they also carry on at least some exchange directly with the water. A similar arrangement exists at the higher trophic levels. Each of the groups of organisms contributes some isotope to the sediments, either through death or via fecal pellets, with bacteria playing an important role in decomposition. Some of the isotope remains with the sediments and, in time, is buried deeper. However, much of it is released back into the water and recycled through the biota.

Figure 5



In many fresh water communities the greatest mass of material will be made up of the photosynthetic plants - the primary producers which constitute the first trophic level. The mass of successive trophic levels will usually be smaller and smaller, so that relatively few carnivorus fish are supported at the top of the biomass pyramid. However, in some open-water communities where the primary trophic level is made up almost exclusively of phytoplankton, with a rapid "turnover", a part of the pyramid may be inverted, (32). Of the total inventory of radiophosphorus and most other radionuclides, one would expect to find the greatest fraction held in those organisms which make up the greatest portion of the biomass. In fresh water systems this will usually be the green plants, which also have rapid exchange rates and an affinity for a large variety of nuclides. Nevertheless, only a small fraction may be held in the large

fish, this may well be the part of greatest interest to the radiation protection specialists.

It should be evident then, that radioisotopes of biologically essential elements, when introduced into streams or lakes, are not apt to remain exclusively in the water. Rather, they will rapidly become distributed between the water, the biomass, and the sediments: In many cases the portion which is removed from the water will exceed that which remains. If it is desired to reduce the specific activity of a contaminating radionuclide by adding carrier to the water, consideration must be given to the probability that there is a greater reservoir of essential elements in the solids (including the biota) than is dissolved in fresh water.

SEASONAL VARIATIONS

Where continued or repetitive release of radioactive waste occurs, a true equilibrium in distribution of radionuclides between water and organisms will seldom be attained. Even under stable conditions of flow and effluent discharge, seasonal fluctuations in the composition and metabolism of the community will keep the system in a dynamic state. New blooms of plankton or vascular plants will create a demand which will draw material from the water and from other parts of the biomass. Conversely, the dying-off of large masses of vegetation may release significant quantities of radionuclides back into the water where they will be available to other components of the community.

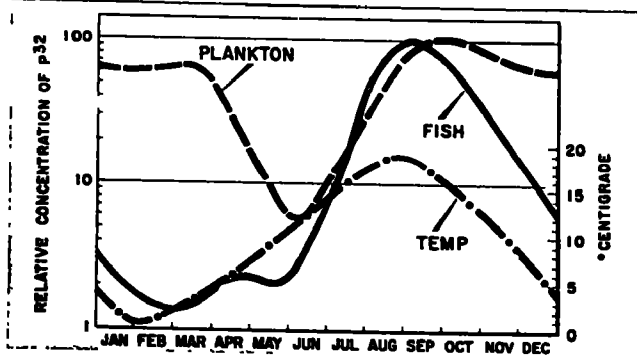
Most aquatic animals are cold blooded, and thus their metabolic rates change with variations in temperature and so, with the seasons. Concentrations of those radionuclides affected by metabolic rates in aquatic organisms will also fluctuate with the seasons. During the cold-water period food intake is drastically reduced. If ingestion is the principal mode of uptake of a radionuclide, the supply to the animal will likewise be reduced, and the quantity of nuclide in the animal will diminish as a result of radioactive decay and biological elimination. The reverse is true when temperatures are high.

Figure 6 shows a smoothed curve for the activity density of plankton and small fish of the Columbia River over the period of a year. Fluctuations in the plankton (predominantly diatoms), are quite similar to those in the water since there is a rapid exchange of nuclides between the water and the plant cells by direct absorption and adsorption; temperature has little effect in this case.

A pronounced decrease in the activity density of the plankton occurs during the late spring, because the flow of the Columbia River increases greatly at this time to provide additional dilution of the effluent from the reactors. Fluctuations in the activity density of the fish are on the other hand, more closely related to temperature. As indicated above, the marked increase in activity density of the fish during the time of high temperature reflects an increase in metabolic rate all along the food chain. Not only are the fish eating more food, but each food organism has become more radioactive, and the effective retention time for radioactive decay within each trophic level has become less. The effect is thus cumulative along the food chain.

Figure 6

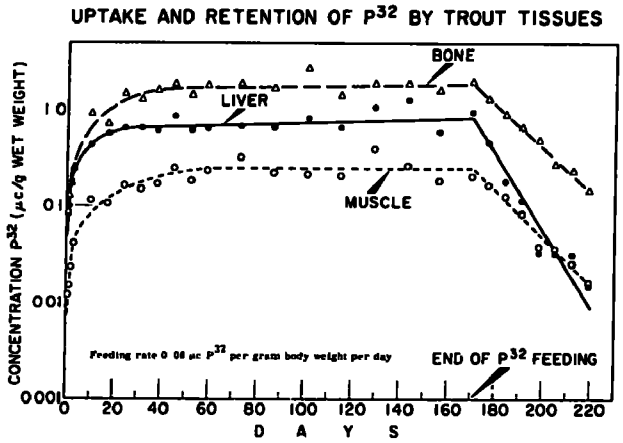
SEASONAL VARIATIONS IN THE CONCENTRATION OF P^{32} IN COLUMBIA RIVER PLANKTON AND FISH



Because of the rapid exchange of some nuclides between the water and algal cells, the activity density of these organisms will rapidly reflect changes in the concentration of the radionuclides in the water. However, the uptake and elimination of most nuclides in the larger forms, such as the fish, is a comparatively slow

process. Their activity density will then fluctuate in a rather sluggish manner in comparison to changes in the water or in their food. Figure 7 shows that trout fed a ration of P^{32} each day required about 2 weeks to reach an equilibrium level. The activity density of the fish may not therefore, provide a good indication of the conditions in the water at the time fish are collected. What may be more significant however, is that the fish samples may provide a reasonably good index of the average conditions which have existed in the water over a period of hours or even weeks, dependent on the particular nuclide involved.

Figure 7



Watson, George, and Hackett, U.S.A.E.C. Document HW-59500 (1959)

CONCLUSION

Radionuclides which enter fresh water streams and lakes may enter the food chain and thus appear in fish and other aquatic forms eaten by man. The radioactive contamination levels which are apt to result in the various organisms, are affected by a large number of physical, chemical, and biological factors. For radioisotopes of some of the more abundant essential elements, we can estimate within broad limits what the maximum levels may be. The characteristics of fresh water communities vary so greatly between different localitits however, that the continued release of substantial quantities of radionuclides must be carried out with considerable prudence and with monitoring of the concentrations of radionuclides which actually result in the environs at the point of release.

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This outline was prepared by R.F. Foster, Manager, Aquatic Biology, Biology Operation, Hanford Laboratories, General Electric Company, Richland, Washington. The report is based on work performed under contract No. AT(45-1)-1350 for the U.S. Atomic Energy Commission.

Additional plates by L.G. Williams, Former Aquatic Biologist, Aquatic Biology Section, Basic and Applied Sciences Branch, Division of Water Supply and Pollution Control.

FWPCA RESPONSIBILITIES FOR WATER QUALITY STANDARDS

I INTRODUCTION

The Water Quality Act of 1965 provided for the most forceful program yet devised to protect and enhance the quality of the Nation's waters. Under this law, the States had until June 30, 1967, to adopt water quality criteria for their interstate and coastal waters and a plan for implementing and enforcing the criteria adopted. Criteria and plans acceptable to the Secretary of the Interior become the water quality standards applicable to the State's interstate and coastal waters.

In the event any State standards are unacceptable, the Secretary of the Interior has the authority to establish standards, but only after the States and all other affected interests have had a full opportunity to be heard. Once adopted, standards are enforceable by the States and the Federal Government if any discharge of material occurs which reduces the quality of the water below the established standards.

The standards make it possible for municipalities, industries, and other water users to know in advance what their responsibilities are for keeping clean waters clean and for restoring polluted waters to a reasonable degree of purity. The standards also give the Federal Government authority to prevent pollution before it occurs, instead of instituting enforcement action after health and welfare are proven to be endangered.

The standards are not intended to "lock in" present uses of water, nor to exclude uses not now possible. Nor are they intended to be least common denominators of water quality and use. Instead, they are intended to enhance the quality and productivity of the Nation's water resources in an orderly, programmed manner.

Standards prepared by the States were submitted and reviewed by the Administration prior to submitting them to the Secretary. The standards-setting effort is the Federal Water Pollution Control Administration's top priority program.

When standards are reviewed, the respective types of water uses determined to be desirable are evaluated against the water quality criteria prescribed to achieve those uses. Close attention is also paid in the review to plans prepared for implementing the standards and to surveillance programs developed for assessing effectiveness of the proposed program.

To assist States in delineating factors to be considered in reviewing standards, the Federal Water Pollution Control Administration prepared and widely distributed "Guidelines for Establishing Water Quality Standards for Interstate Waters" and "Necessary Supporting Material and Implementation Plan Contents." To assist him in evaluating State submissions, the Secretary of the Interior appointed a variety of experts to the National Technical Advisory Committee, with the request that they prepare water quality criteria for water uses in the area of their competence. Subcommittees were established to develop criteria in the following areas: fish, other aquatic life and wildlife, municipal water supplies, industrial water supplies, agriculture, and recreation and aesthetics.

A comprehensive report has been completed and is available in limited quantities.

Standards submitted for review have generally been of a high caliber and represent a substantial Federal-State-local cooperative effort. The standards-setting process has resulted in actions not visible on the horizon initially. Specific programs with a definite schedule have been prepared for cleaning up rivers, lakes, streams, and coastal waters; State laws to permit compliance with the Water Quality Act have been revised where necessary; and information previously unavailable or in an unassembled form has been developed and collected in a single set of documents available to all groups with an interest in preserving and protecting water quality. Most important, however, is the spirit aroused in the preparation of these blueprints for clean water. Officials at all

levels have been surprised at the interest expressed by citizen groups and have themselves become actively involved in assuring the program's success.

Significant as they are, standards are basically a guide for obtaining the longer range objective of improving and protecting water quality. Standards run through the clean-up program, holding together and guiding efforts in planning, waste treatment works construction, research, enforcement, pollution surveillance, training, and technical assistance.

Standards have been partially or wholely approved for all States, three territories, and the District of Columbia. Present efforts are to effect resolution of unacceptable portions of the submitted standards and to develop acceptable anti-degradation statements. Development of additional and more definitive standards may be anticipated as the program progresses.

Additional information on this program may be obtained from the Regional Coordinator of Water Quality Standards or the Water Quality Standards Staff, Office of Program Plans and Development, Federal Water Pollution Control Administration, Washington, D. C. 20242.

The act of setting standards or selecting words or numbers to describe permissible water quality ranges is obviously not an end to itself. Surveillance is required to determine if the standards are being met, and enforcement may be necessary if cleanup operations designed to meet standards are not proceeding on schedule.

The mission of the water quality surveillance program is:

A To monitor water quality and waste facility information for compliance or non-compliance with water quality standards and to report these findings to those in a position to take action.

B To provide the specific information required to assess pollution problems and to evaluate the effects of remedial actions by:

   1 Acting as a support service to other FWPCA programs;

   2 Providing technical assistance to the states;

   3 Cooperating with other Federal agencies and states in the collection of pollution-related information.

C To supply water quality and related pollution information for basin-wide water pollution control planning, to document pollution control violations for enforcement actions, and to fulfill responsibilities as assigned to the program.

The highest priorities for all organizational elements responsible for this program are the planning, developing, and coordinating of the surveillance systems necessary to determine the compliance or noncompliance of the water quality standards and implementation plans.

This will require that Headquarters provide technical guidance to ensure that all data gathering and evaluation are accomplished in a uniform and efficient manner. Particular attention will be given to improving the systems for the collection of water quality data and the establishment of an Early Warning System which identifies trends in water quality in advance of the potential violation.

It will be the responsibility of Headquarters to identify water quality surveillance program activities as they are presently handled in other programs so that these may be consolidated and that sufficient funds and personnel are allocated from these programs to adequately continue the level of surveillance.

Regions will plan and design their surveillance systems on a major basin basis ensuring that the information systems output is current and accurate.

Efforts in information collection, evaluation, and dissemination must be highly coordinated with appropriate Federal, state, local, and private groups or agencies to avoid duplication of effort, to ensure proper allocation of resources, and to consider alternative methods or courses of action.

Each region will provide support for the National effort in analytical quality control in sample handling and analysis. All essential data developed in the region will be put in a storage and retrieval system that is accessible to all of FWPCA.

The order of priority for work assignments in this program will be:

A Water Quality Standards

B Enforcement Activities

C Pollution Control Planning

D Waste Facilities Inventories

E Others as Required

The word "surveillance" suggests a possible observance or inspection of activities. Surveillance as practiced by the Federal Water Pollution Control Administration, however, involves the collecting of discrete information expressly to define the status of water pollution control on a point by point basis so that timely evaluation and remedial action can be effected.

Water pollution control in the United States was launched as a permanent, national program in 1956 with the enactment of Public Law 84-660, the Federal Water Pollution Control Act. Some entirely new concepts were created by the Water Quality Act of 1965 and the Clean Water Restoration Act of 1966. In several respects the recent legislation bears significantly on the water pollution surveillance activity in the United States.

The Clean Water Restoration Act of 1966 transferred administration of the Oil Pollution Act of 1924 from the Secretary of the Army to the Secretary of the Interior. In the process, the Oil Pollution Act, which was formerly limited to vessel pollution affecting coastal navigable waters and the sea within the territorial jurisdiction of the United States, was extended to inland navigable waters and adjacent shorelines subject to pollution from boats and vessels. This new authority complements and expands upon previous responsibilities vested in the FWPCA by PL 84-660, as amended, for the control of pollution resulting from land-based sources, including oil pollution from shore-based facilities.

II SURVEILLANCE ACTIVITIES

A Indices

The fresh waters of our nation must be put to various uses. Among these, municipal water supply has number one priority. As a consequence, statistics are kept on the location, quantity, and type of water treatment provided by every municipality in the United States. Similarly, inventories are maintained of sewerage systems (including waste treatment facilities) delineating the populations served, the type of treatment provided, and the additional facilities needed. National data are also collected, evaluated, and published periodically on the financing and construction of sewage collection and treatment facilities and on pollution-caused fishkills. At present, the major unknowns, insofar as waste discharges are concerned, include those associated with industrial manufacturing, acid mine drainage, thermal pollution, and irrigation return flows. In appreciation of this, the Secretary of the Interior announced that the FWPCA will initiate an industrial waste discharge inventory as a continuing program.

B Water Quality Data

In the final analysis, progress in the field of water pollution control will be measured by the presence or absence of desired levels of quality in the streams and waterways of the Nation. Thus, while the aforementioned statistics are essential, Federal surveillance resources must be aimed primarily at monitoring in-stream conditions for immediate relation to the established water quality standards--point by point, stream by stream, and basin by basin on a day-to-day basis.

To carry out this mission, the FWPCA's Division of Pollution Surveillance pursues the following major stream quality oriented activities via a central facility and regional laboratories.

1 Water Quality Compliance System - Flexible basin-wide systems of water quality monitoring stations, including both central laboratory analyses and in-stream analyses via automatic equipment.

2 Oil Pollution Act Laboratory Services - A unit at the FWPCA laboratory in Cincinnati specializing in techniques required for the detection and analysis of petroleum compounds.

3 Specialized Analytical Services and Instrumentation - Expert analytical services and specialized analytical equipment for national consultation and services and the development, installation, and operation of appropriate automatic water quality sensing and transmitting instruments.

4 Analytical Quality Control - To assess and assure the reliability of laboratory and field analytical processes and results, including the field testing of new laboratory analytical procedures, and the initiation of a complementary program with state and interstate water pollution control agencies to assure the validity of data used in litigation.

5 Data Operations, Evaluation, and Control - To provide and operate a computerized system for the timely storage, retrieval, processing, and analysis of necessary water quality data and related statistics--including interstate water quality standards and implementation plans.

C Surveillance Stations

Aside from the many sampling stations established for limited duration as part of special studies and investigations, FWPCA has, since 1956, established stations at critical points in major basins of the United States. Each of the basic stations is being critically reviewed as to location, collection frequency, and the parametric analyses which will be required in determining compliance with specific water quality standards. Over 100 of the stations are located at or near international or interstate boundaries. Other stations are located at or near the current or proposed municipal water intakes of such major cities as Kansas City, St. Louis, Cincinnati, Washington, Philadelphia, Buffalo, and New York City. Stipulating the development of standards aimed at protecting and enhancing water quality at water supply intakes, many stations obviously must also be located on interstate streams below major municipal and industrial complexes.

Under the terms of an interagency agreement with the U. S. Geological Survey, the Survey will operate these critical long-term monitoring stations. The Survey's responsibilities include sample collection and analysis and entry of the data in the STORET System. FWPCA's responsibilities include continuing review and evaluation of the data to determine adequacy of station location and parameters, frequency of sampling, and the detection of trends or standards violations.

1 Number - It is currently impossible to forecast the eventual requirements for either state or Federal pollution oriented monitoring. We do know that the number of stations must be substantially increased in each major basin to fulfill the Federal responsibility as well as give technical assistance to the states and to permit the Secretary to act quickly if a state fails to establish or maintain satisfactory interstate water quality standards. As in the case of the basic surveillance stations, all new stations including reactivation of stations occupied for short periods during previous investigations, will be scrutinized to assure that they conform to certain basic criteria.

2 Locations required - The locating of the Federal water quality surveillance stations will be influenced by the state implementation plans. However, it is quite predictable that Federal stations will be located at the mouths of rivers and tributaries, the points at which streams cross state lines, points above and below major municipal and industrial complexes, and points designated for specific water uses, including water-based recreation and propagation of fish and wildlife. Some station locations may be thought of as permanent

installations inasmuch as it may be impossible to predict the time when data will not be needed for that point. Conversely, a significant number of stations may measure progress toward water quality objectives which can be met within a predictable, definite period of time. For example, a station established to monitor a point downstream from a specific waste problem may well be deactivated, or at least curtailed, once the problem is ameliorated.

3 Monitoring criteria - In order to maximize the results of vested resources, pollution control monitoring systems must be designed for both flexibility and specificity in regard to sampling locations, analyses performed, and the frequency of analysis. Water pollution surveillance systems must be directed primarily toward detecting changes in the degree of water pollution associated with man's activities. These changes occur with relative rapidity as compared with changes associated with most natural phenomena; for example, the changes occasioned by the passage of water over or through the ground. A new industrial complex, a new sewage treatment plant, or any new water resources structure can effect rapid changes in water quality; hence, the basin system and its component monitoring stations must be periodically reviewed to ensure flexibility to respond quickly to such physical changes. Each year such reviews will undoubtedly result in the relocating of a number of stations, curtailing sampling at some, and expanding the sampling frequency and diversity of analyses at others. Thus, each basin system must be designed to detect and assist in the control of waste discharges, to determine the effectiveness of waste treatment and control, and to measure pollution abatement progress.

4 Parameters required - Although the adoption of an identical, inviolate list of parameters to be associated with each surveillance point would simplify the administration of each basin surveillance system, such rigidity would be neither useful nor desirable. Surveillance for water pollution control purposes will be oriented to specifically stated water quality criteria. Water pollution problems of varying severity exist in most rivers and streams of the United States; yet they are never identical. The surveillance program will be tailored to the very special set of circumstances and needs associated with the specific problem at individual locations. In this sense, the water quality criteria received from the states show a diversity of parameters and serve to define those which are particularly significant for major reaches of interstate waterways. Thus, the parameters requiring analyses may range both within a basin and on a national basis. They may vary from readily obtainable onsite information on dissolved oxygen, conductivity, pH, and temperature to the sophisticated analyses of numerous biological entities, a growing number of radionuclides, toxic substances such as arsenic and heavy metals, plus a formidable array of bacteria, viruses and individual organic contaminants including pesticides and petroleum-based materials, all requiring detailed laboratory evaluation.

5 Frequency of analysis and automatic equipment - The frequency of sampling cannot be irrevocably fixed for an entire system of surveillance stations. Indeed, it cannot be fixed permanently for a single station. Periodic adjustments of frequencies will be necessary for a variety of reasons. The lowest sampling frequency capable of providing desired information is the best frequency. Automatic sensors can facilitate the collection of large amounts of data at closely spaced time intervals and can be used advantageously for a variety of purposes. There is a definite place for such equipment, and there will be more need for it as the water quality standards are implemented. However, each situation will be examined critically to determine if instrumentation is really needed. For example, many physical and chemical characteristics of surface water remain relatively constant, or

change slowly. For such parameters, laboratory analyses of periodic grab samples often are sufficient. Conversely, a critical water quality parameter, such as dissolved oxygen, which is vital to fish and other aquatic life as well as being generally indicative of water quality conditions, may vary under certain conditions from an acceptable to a non-acceptable value in a short time. In general, instrumentation will be applied only to those situations where the parameters are subject to quick change, are essential to the development and verification of mathematical models for evaluating alternate pollution control programs within a basin, or are required to "sound an alarm" at a critical location. The guiding principles in selecting automatic instrumentation, as related to water pollution control, are presented in a "Program Guide for Automated Instrumentation for Water Pollution Surveillance."

III WATER QUALITY MONITORING ACTIVITIES OF THE STATES

Consideration of water quality monitoring activities which can best be conducted by the states, indicates at least three levels of surveillance to be satisfied. First, on-site inspection of municipal and industrial waste handling procedures must be recognized as a basic state or local responsibility; this will assure both more efficient operation of treatment and control facilities and the highest level of return per unit of regulatory effort. Second, monitoring of individual plant effluent lines should be an important part of state programs. Third, the states should examine the receiving water body to assure attainment of desired water quality levels consistent with adopted water quality standards. The latter will require the utmost care to assure coordination and cooperation among the states and the Federal government in fulfilling their mutual responsibilities. Indeed, the need for coordination to assure wise programming of the limited resources of the responsible agency is FWPCA's main reason for not defining the specific stations to be included in each of its basin monitoring systems.

IV ANALYTICAL QUALITY CONTROL

A Need

A principal concern of the FWPCA is the reliability of water quality measurements used to determine compliance with water quality standards. When violations occur, these data will be used to defend litigation proceedings. In anticipation of such circumstances, new emphasis is being given to analytical quality control within FWPCA. The agency and those who work with it must expect court challenges by those who carry the burden of defense. Data which cannot be successfully defended under spirited and argumentative challenges will mean serious loss of state and Federal program effectiveness. Thus, the Federal Water Pollution Control Administration is taking a strong position toward upgrading the present system for analytical quality control and methods validation; before using information from other sources the Administration will scrutinize the data--as it will screen its own--to assure the successful presentation of the facts associated with a specific pollution situation.

V DATA HANDLING

A Coordination

Vital to the FWPCA mission and essential to a coordinated and cooperative state/Federal pollution control effort is a unified or compatible approach to the processing and timely reporting of surveillance data. This includes both water quality information and facilities statistics. Data of the highest scientific accuracy and precision are of little value unless they can be applied at the right time.

B STORET

1 Computer center - In the past, various computer systems have been used to handle the volume of information which must flow among the responsible pollution control agencies. To assist this operation, FWPCA has established the STORET system (STOrage and RETrieval) to assure that all collected information is available in a timely and concise fashion. This system has been converted, along with subsystems now being developed, for use at the Department of the Interior's computer center which utilizes the IBM 360/65.

2 Extent of use - All FWPCA water quality data and related information are currently being placed in the STORET system so that they can be rapidly retrieved to support all operating arms of the Administration throughout the United States. FWPCA invites the use of the STORET system by all water pollution control agencies having need of such data, and FWPCA frequently inserts information developed by other Federal and state agencies if it has a direct use consistent with the pollution control mission. At the present time, STORET contains water quality data and, in many instances, flow data for over 6,000 stations which have been operating for one year or more. At least 3,500 station records include flow data and/or quality data collected by the U.S. Geological Survey which are being used in enforcement, comprehensive planning, and water quality standards development activities of FWPCA. Similarly, FWPCA projects have inserted data from ten states, as collected by state water pollution control or water resource agencies. In addition, water quality and quantity data for over 100 locations have been inserted in STORET to assist the Tennessee Valley Authority to implement pollution control responsibilities.

3 Retrieval of data - Since statistical printouts of STORET data for specific locations are available upon request, many states are now tapping the STORET reservoir of information. The availability of concise printouts, including all available data of reputable quality at a specific location, is particularly valuable in judging whether a proposed criteria will in fact result in water quality enhancement. At present, turn-around time for variable retrievals from the system, as offered to the various agencies, is dependent on the mails. Ultimately, FWPCA is planning a national communications data handling system which would further reduce the time required to respond to requests for services. The cost of such a system will be small as compared to the cost of developing analytical results and will be minor when one considers the estimated 20 to 50 billion dollar cost of the remedial construction program. By connecting the central computer with terminal facilities located in FWPCA regional offices, laboratories, and other major program offices, it will be possible to utilize the full power of the STORET system. Such a network will make STORET and the developing subsystems available to all elements of FWPCA and other water pollution control agencies.

VI SUMMARY

In this new era of water pollution control in the United States, the fight to restore water quality in our environment has just begun. Accordingly, the necessary operations are still in the formative stages as related to the ultimate needs. The type of information to be gathered must be carefully selected; the data must be verified to assure that they meet their intended purpose. The coordinated exchange of information among cooperating and responsible agencies will play a major role in minimizing the cost of surveillance activities as well as speeding water pollution control toward its ultimate goal--enhancement of the quality and value of the Nation's water resources.

This outline was prepared for the Training Program by M. L. Wood, Director, Robert S. Kerr Water Research Center, Ada, Oklahoma.

MARINE AND ESTUARINE PLANKTON

I INTRODUCTION

In recent years the rate of increase in the human population of the earth and the resulting need for more food has intensified our interest in, and increased emphasis on, studies of those organisms forming the key units or "links" in oceanic food chains, the plankton. In addition to this interest, the value of these planktonic organisms as "indicators" of the origin of water masses, both oceanic and estuarine, has become increasingly evident to students of oceanography and estuarine hydrography.

The common organisms making up the estuarine and marine plankton and the general environmental factors influencing their occurrence, abundance and distribution are, therefore, worthy of the attention of all persons interested in the biology of these environments.

II THE ESTUARINE ENVIRONMENT

A Definition

An estuary may be variously defined. For biological purposes it might best be considered as a region where river water mixes with, and measurably dilutes sea water; or, as those water masses which, by virtue of their position, are directly subject to the combined action of river and tidal currents.

B General Structure

1 Physical, chemical and environmental characteristics of an estuary are dependent on the degree of mixing and on the currents and circulation patterns set up when two dissimilar water masses meet.

2 Types of estuary:

a Two-layered

b Partially mixed

c Completely mixed

3 The mixing and circulation of estuaries are controlled by such varied factors as wind, rate of fresh-water run-off, tidal amplitude and physiographic features such as the slope, width and depth of the estuarine basin.

4 These diverse factors acting simultaneously make each estuary a unique constantly changing environment.

III THE ESTUARINE PLANKTON

A Plankton Types

1 Permanent estuarine plankton is composed of those organisms remaining for an appreciable length of time within the estuary.

2 Transient estuarine plankton is made up of those transitory forms from:

a Fresh water feeding into the head of the estuary.

b Marine waters brought into the estuary from the sea.

The stay of transient forms in the estuary is brief and may be limited to a single tidal cycle.

B Permanent Estuarine Plankton

1 Example: St. John River, New Brunswick, Canada

a Salt water penetrates 25 miles upstream from the river mouth.

1) At the upper end of the estuary barnacle nauplii and Sagitta elegans are found in August.

2) Both of these planktonic forms are less common near the mouth of the estuary.

b This is an instance where the length of the estuarine system leads to a water mass and its plankton becoming "stranded" in the system and, therefore, more or less permanent.

2 Example: Miramichi River, New Brunswick, Canada

a Smelt larvae occur in samples from the middle part of the estuary beginning in June.

b During the ensuing summer (June-August) catches in the same area indicate growth of the larvae.

c All evidence indicates a light-controlled diurnal vertical migration of the larvae. In the summer months this would keep them in the bottom layer of inflowing sea water most of the time.

C Transient Estuarine Plankton

1 Example: Margaree River, Nova Scotia, Canada

a This is a very short river as compared with the St. John and Miramichi.

b Plankton tows reveal organisms of marine or freshwater origin depending on state of the tide.

1) At high tide marine cladocerans, copepods, ctenophores, polychaete larvae and marine diatoms are found.

2) At low tide very few planktonic organisms are found and these are all of fresh water origin; e.g., Dephnia, Cyclops and freshwater diatoms.

c No permanent plankton exists in the Margaree estuary, since it is a short, shallow system.

IV THE MARINE ENVIRONMENT

A When compared with that of the estuary the environment of the ocean is found to be more stable.

B Currents

1 No part of the ocean is completely stagnant. Circulation has evolved in all oceans, determined largely by the rotation of the earth, positions of the sun and moon, the winds and the configurations of the ocean basins.

2 There are also currents caused by difference in density, by upwellings and by the entry of river waters into the sea.

C Planktonic distribution is largely a function of temperature, nutrients, salinity, light, and current patterns.

V THE MARINE PLANKTON

A Types of Marine Plankton

1 The phytoplankton includes all the freely floating photosynthetic forms. Individually they are microscopic in size and are unicellular or in groups of cells loosely bound together.

2 The zooplankton includes a great array of micro- and macroscopic free floating animals ranging in size from several micra to about 50 mm. (Some jellyfish may reach 1 m. in diameter.)

3 Marine plankton may be further divided into:

a Temporary plankton consisting of transitory, floating eggs, larvae and juveniles of benthic and nektonic organisms, medusoid stages of benthic coelenterates, and the spores and gametes of various benthic algae.

1) Temporary plankton is often seasonal in occurrence and generally is confined to neretic or coastal waters.

b Permanent plankton consists of organisms completely adapted to open ocean existence which remain floating or feebly swimming throughout their entire life cycle.

B Organisms of the Phytoplankton

1 Diatoms (Bacillariophyceae) are first in rank as producers of organic material (especially in northern waters).

a Reproduction

Reproduction in diatoms is by binary fission. This potential for geometric increase in population size contributes to "blooms" under favorable conditions.

2 Dinoflagellates are generally considered second to diatoms as primary producers though often they are more important in southern waters.

a Dinoflagellates are most well known for their "blooms" which result in catastrophic mortalities. Red tides caused by various species of Gymnodinium in southern areas are a good example.

3 Coccolithophores constitute major components of the plankton in some areas. They are of special geological significance because of the accumulation of their calcium carbonate coccoliths in marine sediments.

C Factors Affecting Distribution of Phytoplankton

1 Light

The amount of light energy available to photosynthesizers diminishes with depth.

2 Vertical circulation

Adequate supplies of plant nutrients (especially nitrates and phosphates) must be present if the phytoplankton is to grow.

a The most abundant source of such nutrients is bottom sediment which is out of reach of the photosynthesizing organisms.

b Methods of returning nutrients to the surface are:

1) Upwellings (most commonly encountered near shore).

2) Divergences of major currents (more common in areas far from shore).

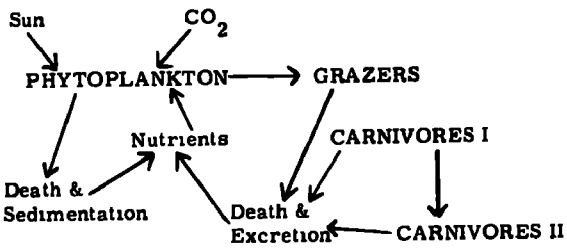
3 Temperature and salinity act largely as selective agents.

D Marine Zooplankton

1 The marine zooplankton contains organisms in every major group of marine animals except the sponges, bryozoans, brachiopoda, and mammals. Copepods are the most abundant representatives.

2 The greatest significance of the zooplankton lies in their role as organic links in the biological economy of the sea.

3 Cyclic Representation of a General Food Chain in the Sea:



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This outline was prepared by G. C. Hughes, Assistant Professor of Biological Sciences, University of the Pacific, and Assistant Professor of Marine Science, Pacific Marine Station, Dillon Beach, Marin County, California.

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	epipholitic, 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

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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This outline was prepared by Lowell E. Keup, Acting Supervisory Biologist, Biological and Chemical Section, National Field Investigations Center, FWPCA, U.S. Dept. of the Interior, Cincinnati, OH.

ARTIFICIAL AND RELATED SUBSTRATES - REFERENCES

INTRODUCTION

This reference list in the field of artificial substrates has been prepared for those not familiar with the literature and for those planning to use one or more of these techniques. It includes references both to periphyton and benthos including techniques, rationale, and practical applications. Since 1967, Dr. C. I. Weber of the Analytical Quality Control Laboratory has compiled an annual bibliography for the Midwest Benthological Society. These bibliographies are recommended for additional and current references. This list is thus complementary to Weber's annual citations for the periphyton. Addresses and sources are given where possible.

Priority has been given to comprehensive works; those of historical note, especially where the original description of a sampler is given, and a sampling of recent papers to show versatility and application for general and specific uses.

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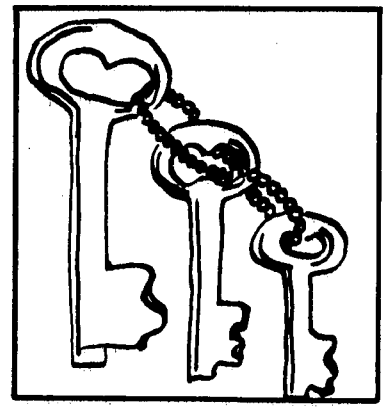
NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Federal Water Pollution Control Administration and the U. S. Department of the Interior.



CHAPTER VII

IDENTIFICATION KEYS

Key to Selected Groups of Freshwater Animals 1

Key to Algae of Importance in Water Pollution 2

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 (1a, 1b), and select the alternative that seems most reasonable. If you selected "1a" you have identified the animal as a member of the group, Phylum PROTOZOA. If you selected "1b", 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 of 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 ANNELIDIA (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, nereid worms. 14
Phylum ANNELIDIA (Fig. 9)

14a Segments with bristles and/or fleshy lobes or other extentions. 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, often slimey, 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 PELECYPODA (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 BRANCHIPODA (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. Cyclops or cladocera.
Subclass CLADOCERA (Fig. 12)

24b Locomotion accomplished by body legs, not by antennae. 25

25a Appendages leaflike, flattened, more than ten pairs.
Subclass BRANCHIPODA (See 22 a)

25b Animal less than 3 mm, in length. Appendages more or less slender and jointed, often used for walking. Shells opaque. Ostracodes.
(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. 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. Mouth-parts 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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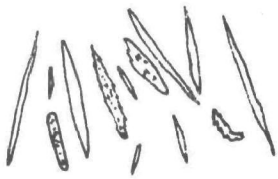
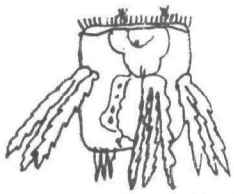


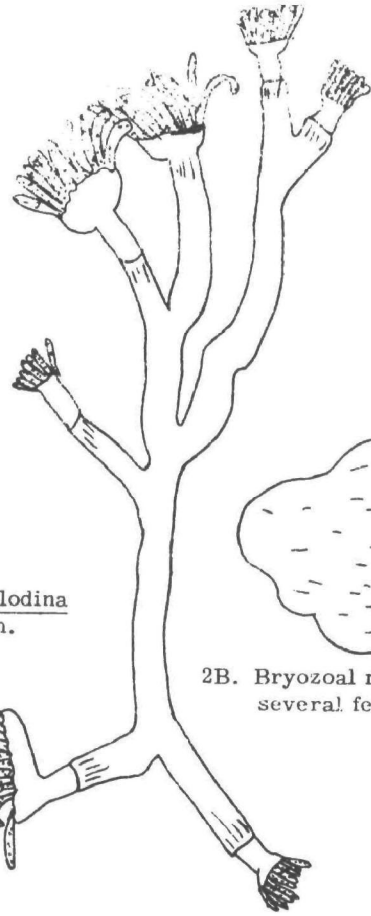
1. Spongilla spicules
Up to .2 mm. long.



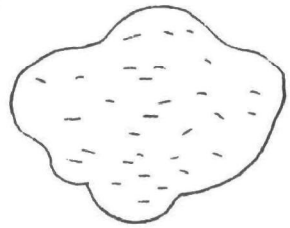
3A. Rotifer, Polyarthra
Up to .3 mm.

3B. Rotifer, Keratella
Up to .3 mm.

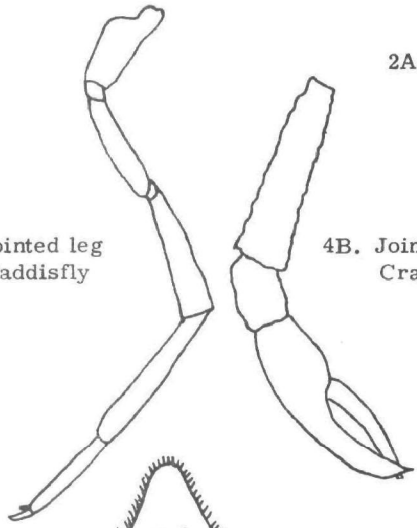
3C. Rotifer, Philodina
Up to .4 mm.



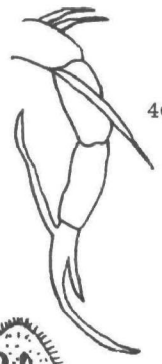
2A. Bryozoa, Plumatella. Individuals up to 2 mm. Intertwined masses may be very extensive.



2B. Bryozoal mass. Up to several feet diam.

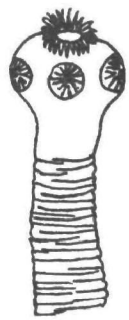


4A. Jointed leg
Caddisfly

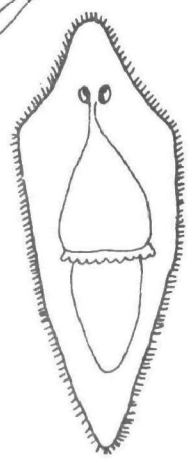


4B. Jointed leg
Crayfish

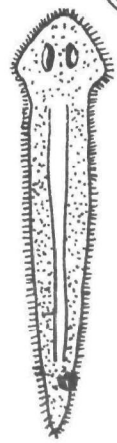
4C. Jointed leg
Ostracod



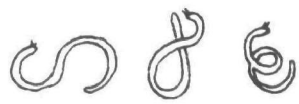
5. Tapeworm head,
Taenia. Up to
25 yds. long



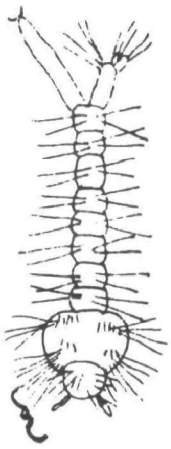
6A. Turbellaria, Mesostoma
Up to 1 cm.



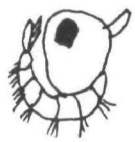
6B. Turbellaria, Dugesia
Up to 1.6 cm.



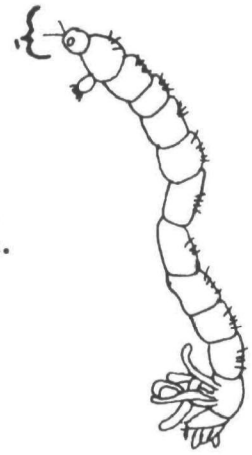
7. Nematodes. Free living forms commonly up to 1 mm., occasionally more.



8A. Diptera, Mosquito larvae
Up to 15 mm. long.

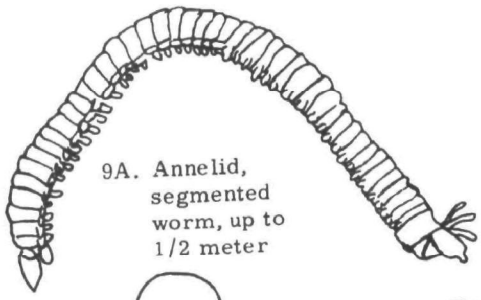


8B. Diptera, Mosquito
pupa. Up to 5 mm.

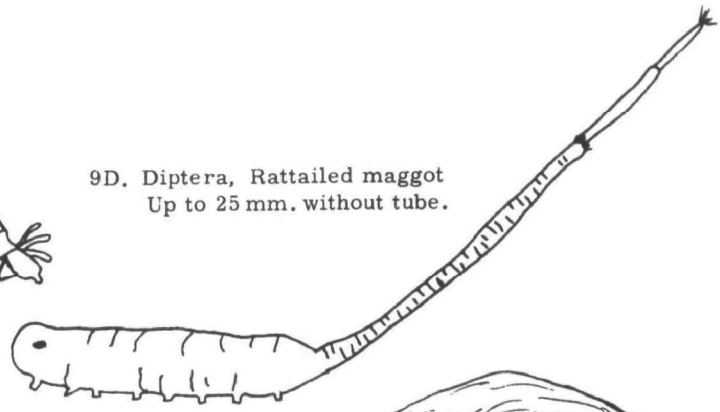


8C. Diptera, chironomid larvae. Up to 2 cm.

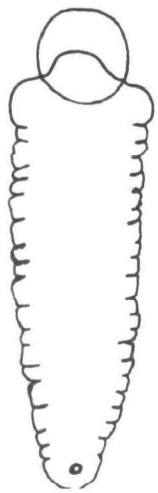
8E. Diptera, crane fly
pupa. Up to 2.5 cm.



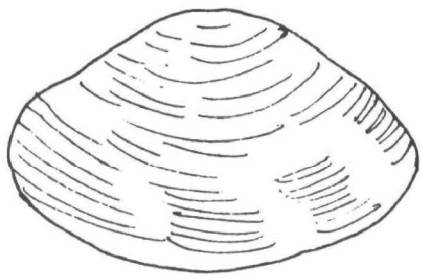
9A. Annelid,
segmented
worm, up to
1/2 meter



9D. Diptera, Rattailed maggot
Up to 25 mm. without tube.



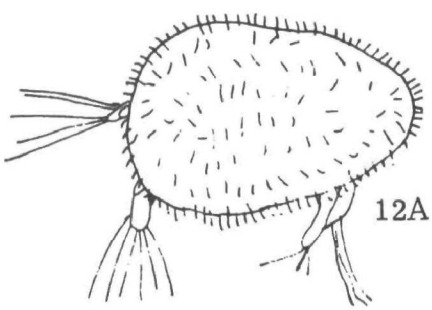
9B. Annelid, leech up to 20 cm.



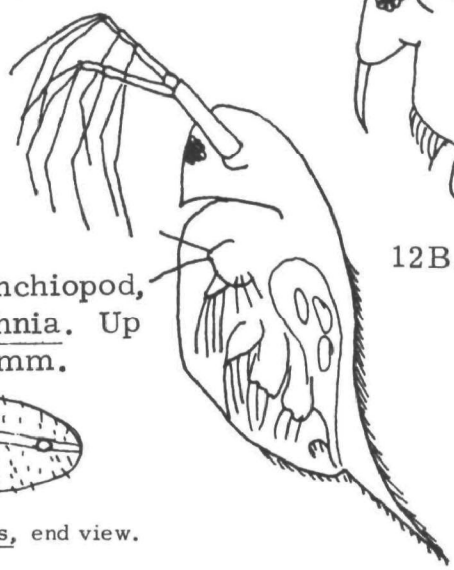
10A. Pelecyopod, Alasmidonta
Side view, up to 18 cm. long.

10B. Alasmidonta, end view.

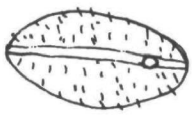
12B. Branchiopod,
Bosmina. Up
to 2mm.



11A. Ostracod, Cypericus
Side view, up to 7 mm.

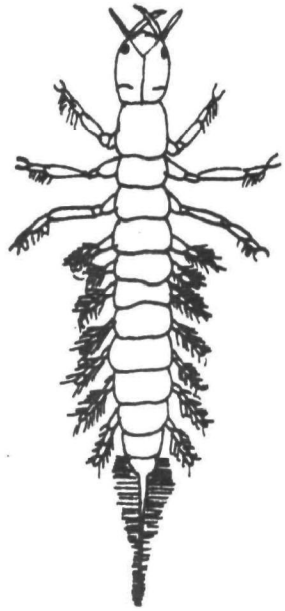


12A. Branchiopod,
Daphnia. Up
to 4mm.

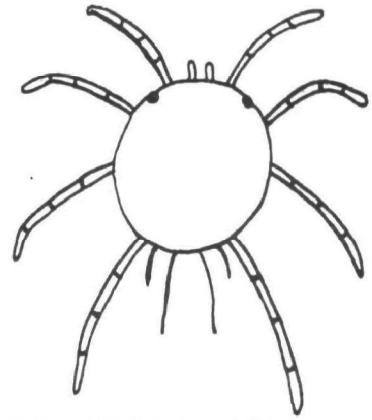


11B. Cypericus, end view.

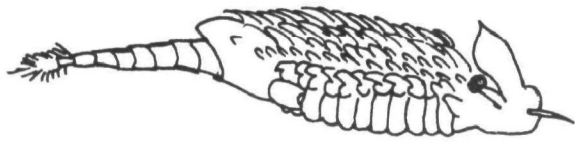
13. Gastropod, Campeloma
Up to 3 inches.



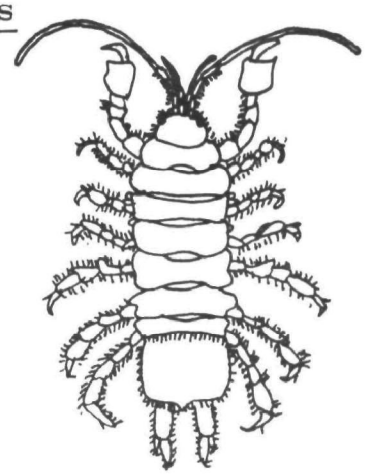
14. Megaloptera, Sialis
Alderfly larvae
Up to 25 mm.



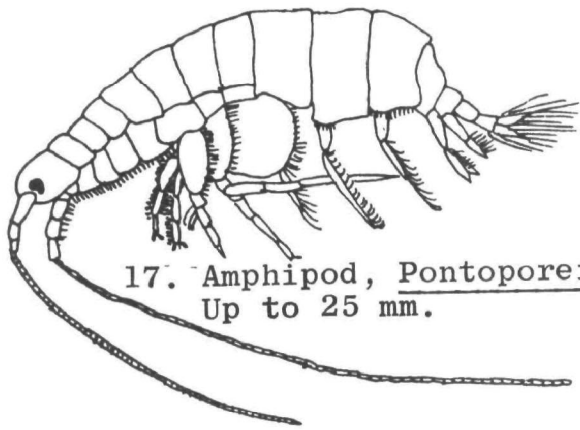
15. Water mite,
up to 3 mm.



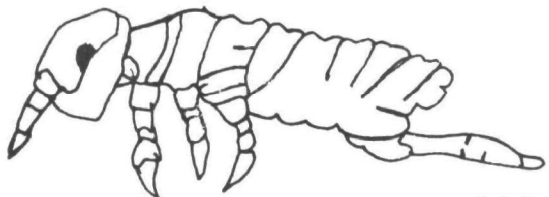
16. Fairy Shrimp, Eubranchipus
Up to 5 cm.



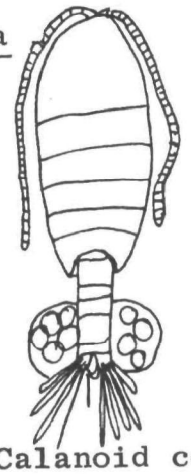
18. Isopod, Asellus
Up to 25 mm.



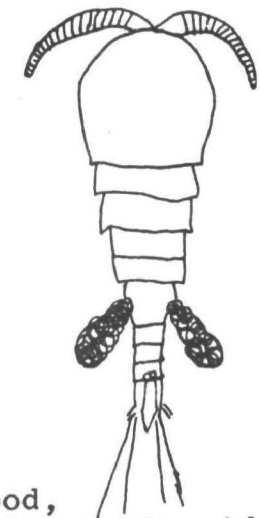
17. Amphipod, Pontoporeia
Up to 25 mm.



20. Collembola, Podura
Up to 2 mm. long



19A. Calanoid copepod,
Female
Up to 3 mm.



19B. Cyclopoid copepod
Female
Up to 25 mm.

21A.

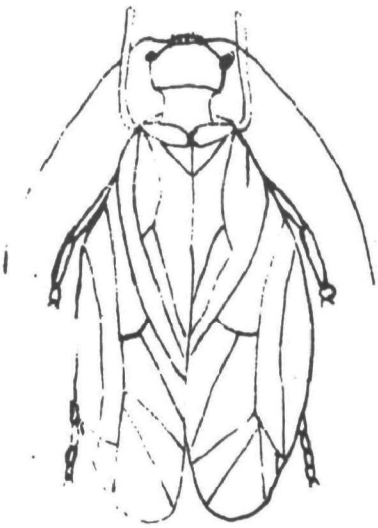
21B.

21C.

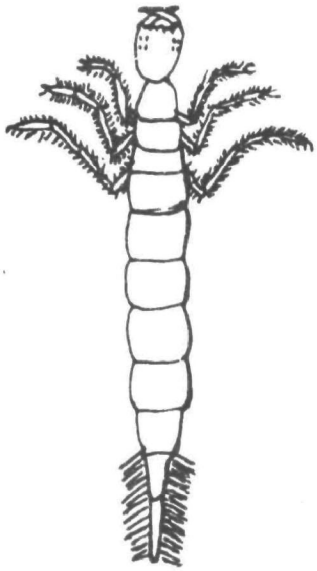
21D.

21E.

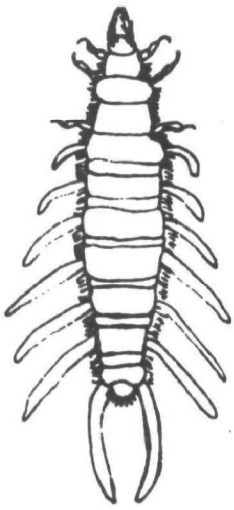
21. Trichoptera, larval cases, mostly 1-2 cm.



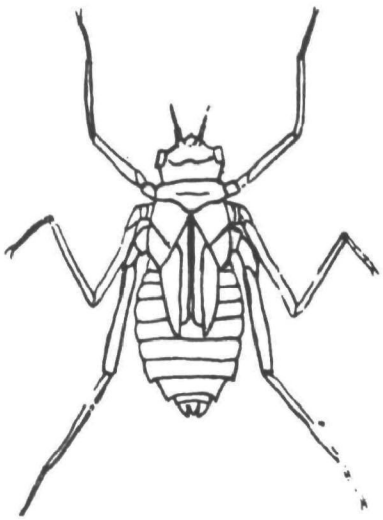
22. Megaloptera, alderfly Up to 2 cm.



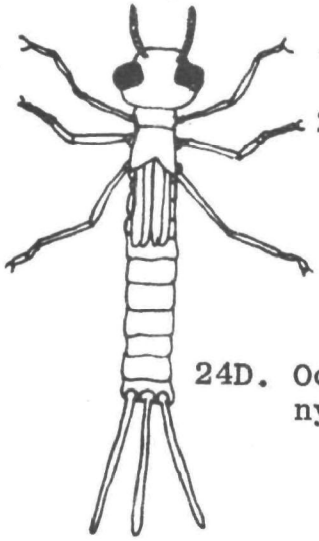
23A. Beetle larvae, Dytisidae, Usually about 2 cm.



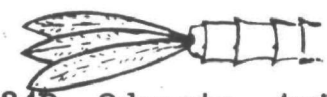
23B. Beetle larvae, Hydrophilidae Usually about 1 cm.



24A. Odonata, dragonfly nymph up to 3 or 4 cm



24D. Odonata, damselfly nymph (top view)



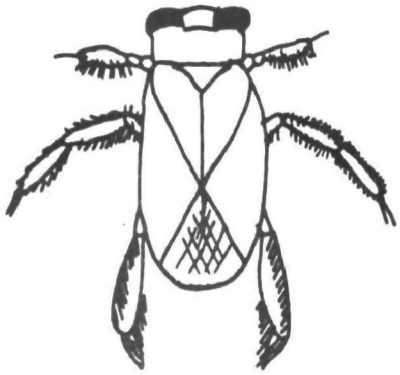
24B. Odonata, tail of damselfly nymph (side view)

Suborder Zygoptera (24B, D)

24E, Odonata, front view of dragonfly nymph showing "mask" partially extended

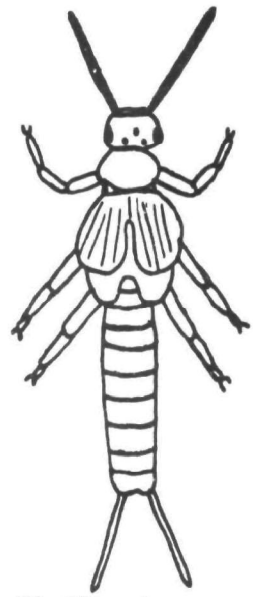
Suborder Anisoptera (24A, E, C)

24C. Odonata, tail of dragonfly nymph (top view)

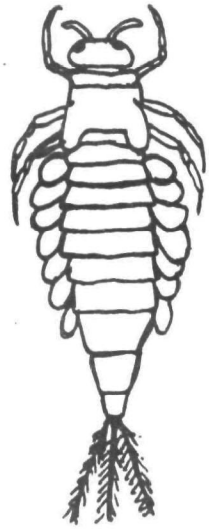


25A. Hemiptera,
Water Boatman
About 1 cm.

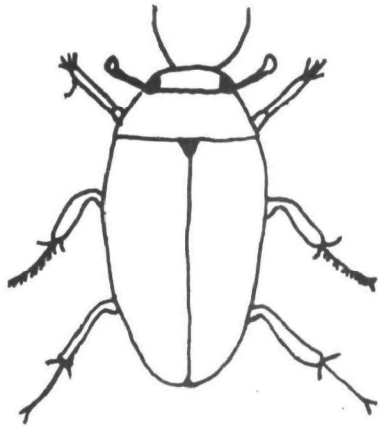
25B. Hemiptera,
Water Scorpion
About 4 cm.



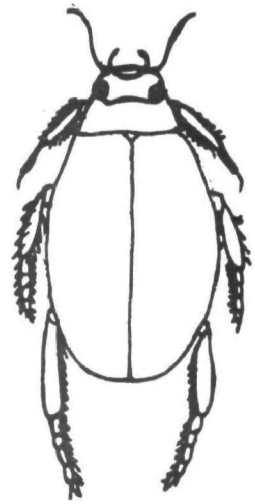
26. Plecoptera,
Stonefly nymph
Up to 5cm.



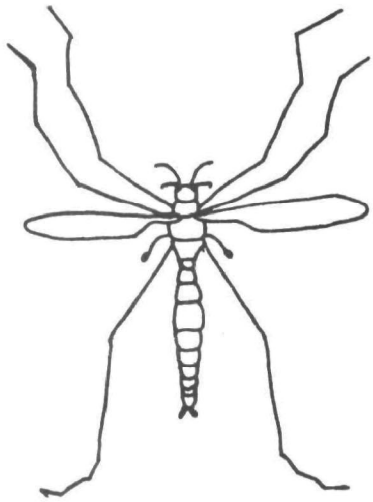
27. Ephemeroptera,
Mayfly nymph
Up to 3cm.



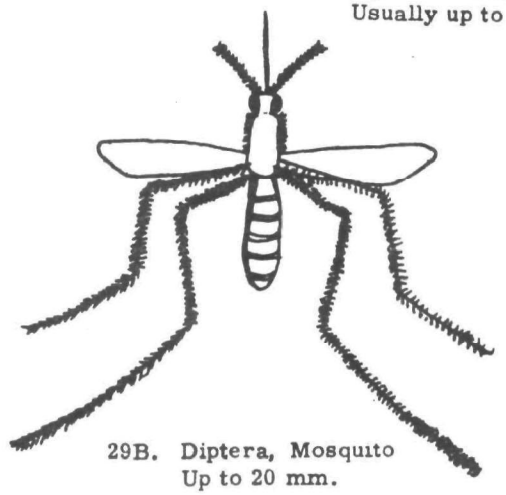
28A. Coleoptera,
Water scavenger
beetle. Up to 4 cm.



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.

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 oedogonia
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 gracilioides
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

APPENDIX

CLASSIFICATION-FINDER FOR NAMES OF AQUATIC ORGANISMS IN WATER SUPPLIES AND POLLUTED WATERS

FOREWORD

The following work is more easily defined in terms of what it is not, than what it is; it is not a "key" in the usual biological sense of the word, nor is it a glossary of a dictionary. It is rather a device for determining what general kind of organism or group is designated by some unrecognized name, be it common or scientific.

If one has access to one or more of the references cited, he can find the same name, and learn much more about it; but not everyone has all of these books, and the information is often couched in highly technical terms.

The nonbiologist would be well advised to read Part I before attempting to use the Finder. The experienced biologist on the other hand may proceed directly to the index and quickly be referred to the larger group to which his unknown organism belongs.

No professional systematist will find himself completely at home. In an effort to present a relatively simple concept of relationships couched in standard terms for all groups or organisms, some violence was done to certain highly sophisticated systems of classification. It is hoped, however, that the layman will find accuracy sufficient for his needs, and the specialist will be referred to technical literature where he can satisfy his needs for greater detail.

While every effort has been made to ensure accuracy, it is inevitable that errors have crept in. Please call them to our attention.

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H.W. Jackson
Chief Biologist
National Training Center

PART I. The System of Biological Classification

PART II. Outline of Biological Classification

PART I

The System of Classification

CLASSIFICATION - FINDER
for
NAMES OF AQUATIC ORGANISMS
in
WATER SUPPLIES AND POLLUTED WATERS

Part I. The System of Classification

I INTRODUCTION

A Every type of living creature has a favorite place to live. There are few major groups that are either exclusively terrestrial or aquatic. The following remarks will therefore apply in large measure to both, but primary attention will be directed to aquatic types.

B One of the first questions usually posed about an organism is: "What is it?", usually meaning "What is it's 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.

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

2 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 subdivisions. There are over a million and a half items (or species) included in the system of biological nomenclature (very few libraries have a million books).

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

a It is based on a system of groups and super groups, of which the foundation (which actually exists in nature) is the species. Everything else has been devised by man and is subject to change and revision as man's knowledge and understanding increase.

b The basic categories employed are as follows:

(1) Similar species are grouped into <u>genera</u> (genus)

(2) Similar genera are grouped into <u>families</u>

(3) Similar familes are grouped into <u>orders</u>

(4) Similar orders are grouped into <u>classes</u>

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

(6) Similar phyla are grouped into <u>kingdoms</u>

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

a 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:

<u>Homo sapiens</u> - modern man

<u>Homo neanderthalis</u> - neanderthal man

<u>Esox niger</u> - Chain pickerel

<u>Esox lucius</u> - northern pike

<u>Esox masquinongy</u> - muskellunge

b 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

RELATIONSHIPS BETWEEN LIVING ORGANISMS

ENERGY FLOWS FROM LEFT TO RIGHT, GENERAL EVOLUTIONARY SEQUENCE IS UPWARD

PLANTS 5	**ANIMALS 81**	**FUNGI 250**
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
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NOTE: NUMERALS REFER TO PARAGRAPHS IN PARTS 2 AND 3.

W. B. COOKE AND H. W. JACKSON, AFTER WHITTAKER

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genus Anabaena (an alga), we must simply use the generic name, and:

Anabaena planctonica,

Anabaena constricta, and

Anabaena flos-aquae

are three distinct species which have different significances to water treatment plant operations.

5 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 frog spittle, a common filamentous alga, and a crayfish or crawdad are shown side by side below. Their scientific names are Spirogyra crassa and Cambarus sciotensis.

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

(Frog Spittle)		(Crayfish)
Plantae	Kingdom	Animalia
Chlorophyta	Phylum	Arthropoda
Chlorophyceae	Class	Crustacea
Zygnematales	Order	Decapoda
Zygnemataceae	Family	Palaemonidae
Spirogyra	Genus	Cambarus
crassa	Species	sciotensis

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 crayfish is as follows:

Kingdom Animalia

Phylum Arthropoda

Class Crustacea

Subclass Malacostraca

Order Decapoda

Section Nephropsidea

Family Astacidae

Subfamily Cambarinae

Genus Cambarus

Species sciotensis Rhoades 1944

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 name for a given species is also sometimes disputed, and so species names too are changed. The species itself, as an entity in nature, however, is relatively timeless and so does not change to man's eye.

II THE GENERAL RELATIONSHIPS OF LIVING ORGANISMS

A Living organisms (as contrasted to fossil types) have long been grouped into two kingdoms: Plant Kingdoms and Animal Kingdoms. 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:

1 Plantae: photosynthetic; synthesizing 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 PROTISTA.

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

a The bacteria and blue algae, lacking a nuclear membrane, may be considered as the lower protista or MONERA.

b The single celled algae and protozoa having a nuclear membrane, are best referred to simply as the higher protista.

PART II

Outline of Biological Classification

Part II. Biological Classification

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