# BIOLOGICAL FIELD INVESTIGATIVE DATA FOR WATER POLLUTION SURVEYS

UNITED STATES
DEPARTMENT
OF THE INTERIOR

FEDERAL WATER POLLUTION
CONTROL ADMINISTRATION

# BIOLOGICAL FIELD INVESTIGATIVE DATA FOR WATER POLLUTION SURVEYS

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

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 Price 70 cents (paper cover)

# ACKNOWLEDGMENTS

The authors gratefully acknowledge the permission granted by Dr. Ralph E. Fuhrman, Executive Secretary of the Water Pollution Control Federation, and Mr. William A. Hardenburg, Editor of *Public Works*, to reprint from their respective Journals the materials which appear herein as Chapters III and IV.

Further acknowledgment is accorded Mr. R. Keith Stewart and Mrs. Martha Jean Wilkey whose diligent and consistent contributions to the organization of text and illustrative material, the several bibliographies, and styling of the typewritten draft effectively shortened the time between initiation and completion of this project.

U. S. DEPARTMENT OF THE INTERIOR
FEDERAL WATER POLLUTION CONTROL ADMINISTRATION
WP 13
JULY 1966

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# PREFACE

A compilation such as this was first envisioned by the senior author in 1950 during the early years of the national water pollution control program, when he served as the first full-time biologist assigned to training activities of the U.S. Public Health Service at the Environmental Health Center, Cincinnati, Ohio.

Over the past 15 years there has been great demand for biological information as related to water pollution prevention and abatement programs, indicating a need to publish under one cover the natural history aspects of water pollution investigations. The authors have responded to numerous invitations to present lectures covering this type of information in departments of engineering and conservation; and all the while there have been continuous requests for prints of the articles here collected—further pointing up the need to develop a volume of this kind.

Today there are many professions working in water pollution control. All of them can well utilize some basic knowledge of the ecological environment. This book will serve to introduce the non-biologist to the life sciences as they relate to water pollution and its control. The professional biologist, inexperienced in water pollution investigations, will find the book a quick introduction to field studies of polluted streams, lakes and artificial impoundments. For the professional investigator, sources of further information, both general and detailed, are set forth in the selected references and bibliography to help in his field studies—an underlying feature of the national effort to conserve and protect our water resources.

*Chapter I**

# HISTORY

## CHRONICLE

Since the turn of the century, and even before, biologists have struggled to determine the impact of civilization's rejectamenta on aquatic biota and to explain these phenomena to their associated disciplines and to the public. The chronicle of published effort began with Hassall in 1850 (1850, 1856) who noted the value of microscopic examination of water for the understanding of water problems. Sedgwick (1888) led in application of biological methods to water supply problems. The Massachusetts State Board of Health was the first agency in the United States to establish a systematic biological examination of water supplies. In 1889 Sedgwick collaborated with George W. Rafter to develop the Sedgwick-Rafter method of counting plankton. Whipple (1899) produced a treatise that, in 1948, was in its fourth edition and fifth printing; it has served through the years as an often-used reference in the water supply and water pollution field.

One of the first practical applications of biological data to the biological definition of water pollution was contained in the "saprobien system" of Kolkwitz and Marsson (1908, 1909). This system, based on a check list detailing the responses of many plants and animals to organic wastes, has been extensively used to indicate the degree of pollution at a given site. That the sound basic judgment of these early investigators has withstood the passage of time is shown by the frequent references currently made to their works.

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*Taken From: "Pollution and The Life in Water," by Kenneth M. Mackenthun and William Marcus Ingram, Public Health Service Publication No. 999–WP–20. pp. i-16, May 1965.

The survey of the Illinois River by the Illinois Natural History Survey was one of the first that clearly demonstrated the biological effects of organic pollution; a series of papers represents studies that provided much impetus and professional status to biological stream investigations in the United States (i.e., Forbes and Richardson, 1913, 1919; Forbes, 1928). Richardson (1921) showed that changes had occurred in the bottom fauna of the Illinois River since 1913 as a result of the increased movement of sewage pollution southward. Later, Richardson (1928) noted that ". . . the number of small bottom-dwelling species of the fresh waters of our distribution area that can be safely regarded as having even a fairly dependable individual index value in the present connection is surprisingly small; and even those few have been found in Illinois to be reliable as index species only when used with the greatest caution and when checking with other indicators."

Purdy (1916) demonstrated the value of certain organisms for indicating areas of the Potomac River receiving sewage discharges. The shallow flats of the Potomac River were found to be of great importance in the natural purification of organic wastes; sunlight and turbidity were observed to be prominent factors in the determination of oxygen levels and in waste purification processes. Weston and Turner (1917), Butterfield (1929), and Butterfield and Purdy (1931) reported other studies that demonstrated the effects of organic enrichment on a stream, the sudden change in the biota after the introduction of the waste, and the progressive recovery of the biota downstream as the wastes were utilized.

Butcher (1932, 1940) studied the algae of rivers in England and noted that attached algal forms gave the most reliable indication of the suitability of the environment of an area for the support of aquatic life. In the United States, Lackey (1939, 1941a, 1942) worked with planktonic algae and noted their response to various pollutants. The work of Ellis (1937) on the detection and measurement of stream pollution, the effects of various wastes on stream environments, and the toxicity of various materials to fishes has served as a reference handbook and toxicity guide through many years.

Cognizance has been taken of the biotic community and the effect of pollution on the ecological relationships of aquatic organisms (Brinley, 1942; Bartsch, 1948). Bartsch and Churchill (1949) graphically depicted (Figure 1) the biotic response to stream pollution and related stream biota to zones of degradation, active decomposition, recovery, and clean water. Patrick (1949) described

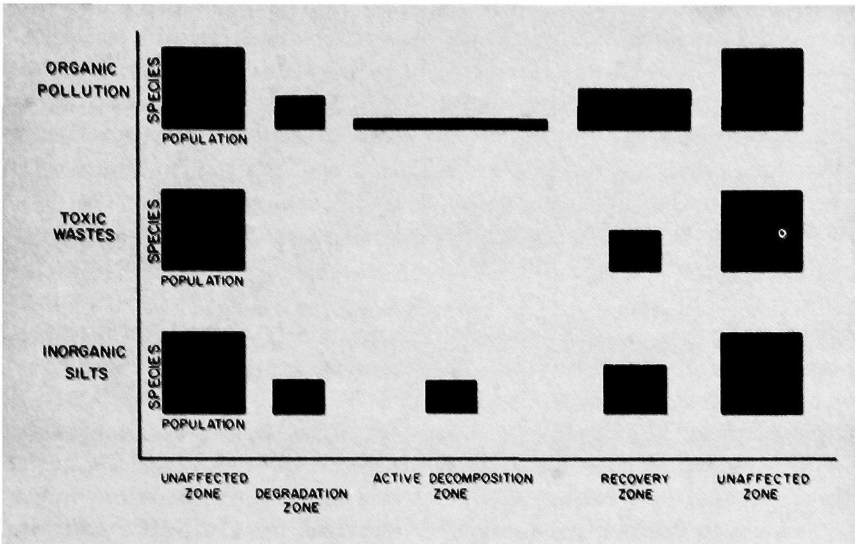


**Figure 1. Response of benthos to pollution.**

a healthy stream reach as one in which ". . . the biodynamic cycle is such that conditions are maintained which are capable of supporting a great variety of organisms," a semihealthy reach as one in which the ecology is somewhat disrupted but not destroyed, a polluted reach as one in which the balance of life is upset, and a very polluted reach as one that is definitely toxic to plant and animal life. Patrick separated the biota into seven groups and demonstrated specific group response to stream conditions by bar graphs. The number of species was used rather than the number of individuals. Fjerdingstand (1950) published an extensive list placing various algae and diatoms in zones or in ranges of stream zones similar to those of Kolkwitz and Marsson.

## ORGANISM RESPONSES

The "classical" benthic organism responses to organic wastes have been detailed frequently in the literature (Hynes, 1960; Biglane and Lafleur, 1954; Hirsch, 1958; Dymond and Delaporte, 1952; Pentelow, 1949; Van Horn, 1949, 1952; Bartsch and Ingram, 1959; and Gaufin, 1958). Benthic organisms are directly subjected to adverse conditions of existence as a result of their preferred habitat and their general inability to move great distances by self motion. Different types of organisms respond in a variety of ways to changes that may occur in their environments. Some species cannot tolerate any appreciable water quality changes, whereas others can tolerate a wide range of water quality, and some very tolerant ones are able to live and multiply under extremely adverse environmental conditions. Generally, a natural, unpolluted stream reach will support many different kinds of organisms but relatively few individuals of a given species because of predation and competition for food and living space. The converse most often exists in a stream reach polluted with organic wastes. In such a reach, most predators are eliminated by water quality or substrate changes, living space presents no problem because remaining organisms must be well adapted to live in organic sludge, and food is seemingly inexhaustible. Sludgeworm populations have, on occasion, been calculated to exceed 50,000 pounds per acre of stream bottom.

Patrick (1953) listed five conditions caused by wastes that may be harmful to aquatic life: dissolved oxygen deficiency, toxicity, extreme temperature changes, harmful physical abrasion, and deposits that render the bottom substratum untenable for habitation.

Gaufin and Tarzwell (1952, 1956) described extensive studies of Lytle Creek, which received organic pollution. In the septic zone it was found that 40 percent of the benthic population was Diptera, 20 percent Coleoptera, 20 percent segmented worms, 10 percent Hemiptera, and 10 percent Mollusca. All insects were characterized by having some means of using atmospheric oxygen. Hawkes (1963) observed that the riffle community is remarkably sensitive to changes in the organic loading of the water, and since organic and mineral matter and organisms are constantly being lost by the streambed community, most stream communities rely on sources outside the stream itself for their basic materials. Butcher (1959) stated that with gross organic pollution the flora of a river consists of "sewage fungus," and the fauna, of tubificid worms and *Chironomus* larvae. As the organic matter decomposes (with increasing distance from the source of pollution), *Asellus* replaces *Chironomus,* then mollusks appear, and finally caddisfly larvae and fresh-water shrimp.

Ingram (1957) discussed the pollutional index value of mollusks and stated that "Apart from systematic morphological studies, it is not realistic to isolate a single group of organisms such as mollusks from other animals and plants that are associated under similar ecological conditions in clean or polluted water. It is the study of the total biota which tells one most about water conditions."

Groups of related organisms have, however, been used to indicate water quality. Palmer (1957) stated that ". . . it appears evident to many workers that particular genera or even species of algae, when considered separately, are not reliable indicators of the presence or absence of organic wastes in water. However, when a number of kinds of algae are considered as a community, that group may be reliable as such an indicator." Lackey (1941b) listed a number of algae that thrive best in polluted water. Patrick (1957) stated that diatoms are a desirable group for use to indicate stream conditions because they need no special treatment for preservation. The diatom flora of a normal stream is made up of a great many species and a great many individuals, and diatoms as a group vary greatly in their sensitivity to chemical and physical conditions of water. She also concluded (Patrick, 1948) that the attached forms give the most reliable indication of the suitability of the environment for the support of aquatic life.

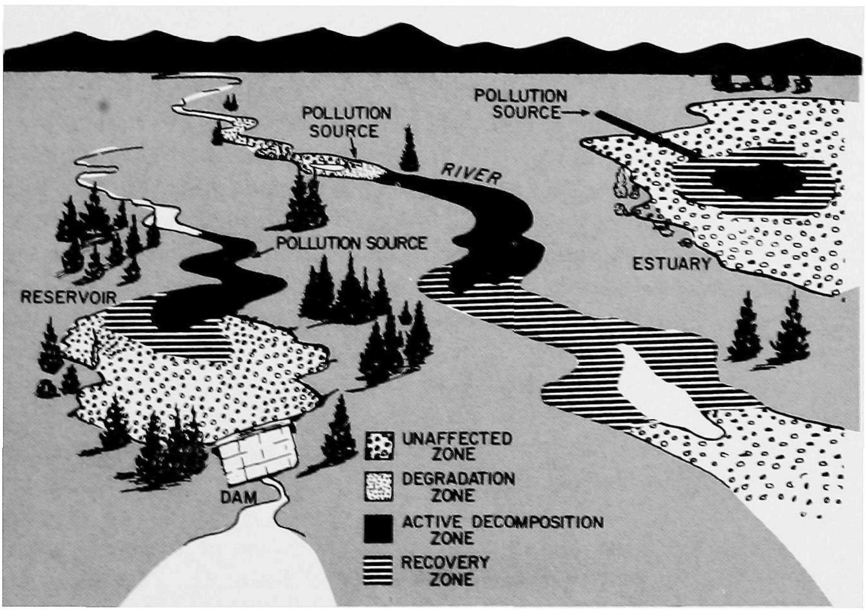


**Figure 2. Benthic zones of pollution (organic wastes).**

Czensny (1949) observed the effects of different types of pollution on fish, on fish food, and on the over-all fisheries resource. Doudoroff and Warren (1957) stated that ". . . only fish themselves can be said to indicate reliable environmental conditions generally suitable for their own existence." Katz and Gaufin (1953) studied the effects of sewage pollution on the fish population of a midwestern stream and concluded that the presence of black bass and darters is good evidence that organic pollution is not a major limiting factor in an area. Mills (1952) stated that the fish population itself is the index or pointer to the other small forms that need to be considered. Katz and Howard (1954) found a significant difference in the length of fish of the same year-class in the various pollutional zones, with the greatest length attained in the enriched lower portion of the recovery zone. In this study, no relation between growth of fishes and volume of bottom organisms was apparent.

Toxic wastes have a severe impact on aquatic biota. Notwithstanding the variation in response to a specific concentration of a toxicant among aquatic animals and plants, a toxic substance eliminates aquatic biota until dilution, dissipation, volatilization, etc., reduce the concentration below the toxic threshold (see Figure 2). There is no sharp increase in certain forms as there is with organic wastes; rather there is an abrupt decline in both species and population followed by a gradual return to normal stream inhabitants at some point downstream. The bioassay is, therefore, an important tool in the investigation of toxic effluents.

The effects of inert silts on the benthos is similar to those of toxic wastes, but usually not so severe. Generally, both the number of species and the total population following silt pollution (Cordone and Kelley, 1961) are depressed. The algal population is also often much reduced from the population occurring in areas not laden with silt.

Lakes and other standing waters do not usually support the variety of benthos found in streams. As with streams, however, organic pollution eliminates many benthic forms and results in population increases among the more tolerant varieties (Surber, 1953). Surber (1957) stated that "A survey of the lake reports showed that an abundance of tubificids in excess of 100 per square foot apparently truly represented polluted habitats." Changes in the benthic population structure are especially evident in the alluvial fans produced in lakes by polluted influent streams (see Figure 2). Along with changes in the benthos, the nutrients contributed by organic pollution may stimulate aquatic growths that will have a severe impact on the recreational use of the water. Resultant algal blooms concomitant with recycling and reuse of nutrients within the lake basin contribute to and hasten inevitable eutrophication.

The estuarine and marine environments have not been studied as extensively as the fresh-water habitats. Reish (1960) cited Wilhelm (1916) to the effect that the polychaete *Capitella capitata* (Fabricius) plays a role in marine waters similar to that the oligochaete *Tubifex* plays in fresh water. Filice (1954) and Reish (1960) found three benthic zones surrounding a major pollutional discharge: one essentially lacking in animals, an intermediate zone having a diminished fauna, and an outer zone unaffected by the discharge. Filice (1959) found the crab *Rhithropanopeus harrisii* (Gould) present more abundantly than expected near industrial outfalls; this crab and *Capitella capitata* (Fabricius) were present in large numbers near domestic outfalls. Hedgpeth (1957) reviewed the biological aspects of the estuarine and marine environments.

## REALITY AND FIELD OPERATIONS

Biological surveys may be tedious, time consuming, specialized, demanding, and sometimes expensive, but they are never monotonous and are seldom routine. Surveys can involve many facets of the aquatic biota or they may concentrate on one group of organisms (see Figure 3). Something that may be termed "reality," equated with the magnitude of the problem, most often dictates the type of study and the kinds and numbers of samples to be collected. To those faced, for example, with an administrative request for a report in 3 weeks on 100 miles of stream with 30 outfall sewers clustered within a metropolitan area, reality dictates the extent and scope of field studies. Biological sampling downstream from each outfall would not be feasible and indeed it would not be biologically possible to distinguish among many of those outfalls in close proximity to each other.

Excluding routine plankton collections, a biologist should always collect his own samples. Nowhere in the sanitary sciences is more sound field judgment required than that required of the biologist in taking his samples and in observing the environment from which the sample came. Much of his field value lies in his astute observation of change within the growth patterns of those biota subject to any adversities within the environment.

Many streams, because of their physical makeup, do not lend themselves to benthic sampling with routine tools such as the Ekman dredge, Petersen dredge, and Surber square-foot sampler. Cooke (1956) reviewed the literature on colonization of artificial

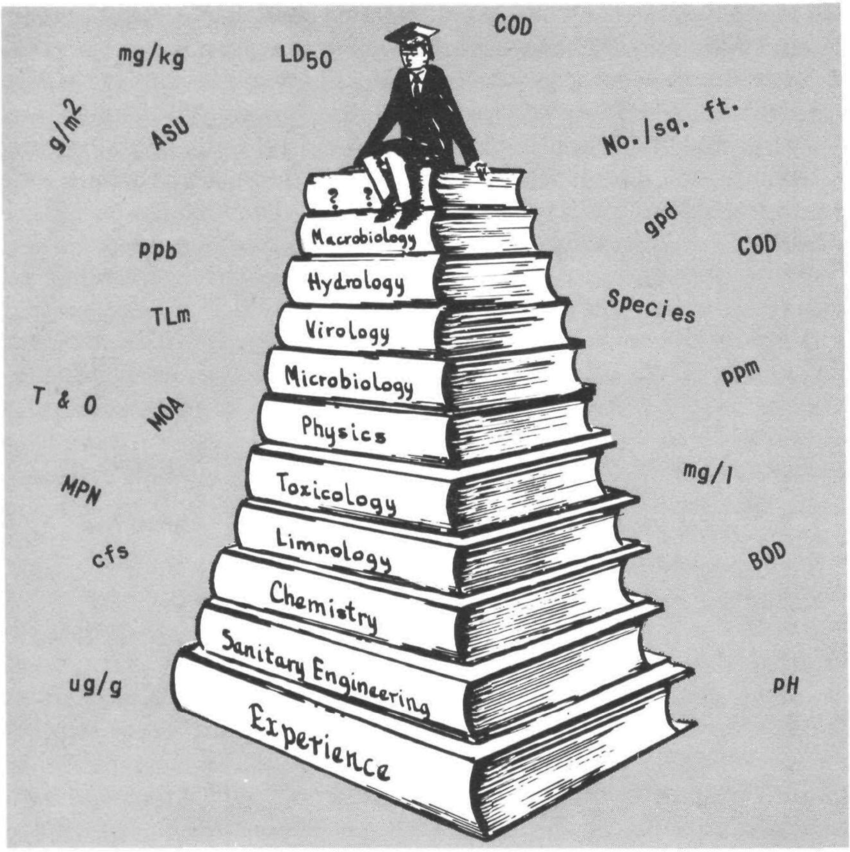


**Figure 3. Pollution evaluation requires a solid foundation supplied only by interrelating many disciplines.**

bare areas; Scott (1958) described sampling with brush boxes in nonproductive stream areas; and Hester and Dendy (1962) described the use of a multiple-plate sampler made from 3-inch masonite squares separated on a rod by 1-inch masonite squares. The multiple-plate sampler has been found to be an effective tool in several streams throughout the United States. Lund and Talling (1957) and Sladeckova (1962) described sampling methods for the algal and periphyton communities. Many sampling procedures and techniques were detailed by Welch (1948). The biologist should relate all routine sampling procedures to *Standard Methods for the Examination of Water and Waste-water* (APHA, 1960), or his report should contain a description of those techniques that differ.

## SELLING THE PRODUCT

Too often the vital massage that biology can bring to the definition of the pollution problem has been lost because of the obscurity of presentations sprinkled liberally with vague generalities and because of lack of understanding and appreciation of the language used to couch the message. Often basic facts become mired in technical explanation. The biologist presently must travel more than halfway if he is to sell the products of his science to the reader. Good, concise, assertive reporting supported by uncluttered, pertinent graphical material does much to please and stimulate the reader to greater comprehension of the findings of fact. One of the biologist's challenges is to present information that is understandable, meaningful, and helpful to associated disciplines, to administrators, and to the general public who are the financial supporters as well as the benefactors of a pollution abatement program.

Recently several methods have been proposed for the presentation of biological data. Beck (1954, 1955) grouped benthic organisms into five classes based on their sensitivity to environmental change and proposed a numerical biotic index that represented a summation of those species that tolerate no appreciable pollution and those that tolerate only a moderate amount. Beak (1963) modified Beck's reporting method to include three groups in which all occurring species are placed: those very tolerant of pollution, those occurring in both polluted and unpolluted situations, and those intolerant of pollution. Points are arbitrarily assigned to each group, and a biological score results from adding the points at a given station.

Wurtz (1955) developed for each station a four-column histogram in which the columns represent basic life forms: burrowing organisms, sessile organisms, foraging organisms, and pelagic organisms. Columns are plotted as a frequency index in which the total number of species found at any station represents a frequency of 100 percent for that station.

Beak et al. (1959) used bivariate control charts to describe changes in benthos adjacent to the site of a large chemical plant. Burlington (1962) statistically calculated a "coefficient of similarity" among stations; for each specific group of organisms, he used "prominence values" that take into account both density and frequency of observation. Patrick and Strawbridge (1963) stated that it is relatively easy to determine the presence of large amounts of pollution, but that the determination of definite but borderline deterioration of water quality is in some cases difficult. They presented a mathematical method whereby the limits in variation of natural populations, especially diatoms, can be defined.

Ingram and Bartsch (1960) pleaded for the use of common, understandable terms in presentations on biology. They pointed out the value of photographs to depict unusual environmental conditions and showed a number of different graphical presentations used in investigational reports.

Serious thought should be given the methods and techniques of reporting data to ensure that the final report meets the needs of the study and provides answers to questions originally responsible for the initiation of the study. Often less thought and consideration are given to reporting data than to collection and analyses of data, even though each is equally important to a successful contribution.

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*Chapter II**

# TERMINOLOGY

ACCLIMATION—The organism's adjustment to a change in an environment.

ACTINOMYCETES—Unicellular, filamentous microorganisms frequently grouped separately to occupy a position between the fungi and the bacteria, although more closely associated with the bacteria. They are widely distributed in nature and account for a large part of the normal population of soils and lake and river muds. Their ability to produce earthy odors has long been recognized.

ACTIVE DECOMPOSITION ZONE—In streams polluted with organic wastes, a zone of active decomposition often follows a zone of degradation. In the zone of active decomposition, the biological oxygen demand (BOD) undergoes partial satisfaction, the D.O. reaches its low point of the curve and may go completely to zero in the upper end of the zone. Sludge deposits attain their maximum depth at the upper limit of the zone, and turbidity gradually diminishes throughout the zone. Molds, fungi, and filamentous bacteria reach peaks in the upper limits of the zone and gradually diminish. Ciliates, flagellates, and bacteria-eating protozoa reach a peak of abundance. Various forms of algae may attain a prolific growth near the lower limits of the zone. Sludgeworms, very tolerant midge larvae, and occasionally leeches reach their peak of production. The population abundance is very high in this zone, and sludgeworm populations have been estimated in excess of 50,000 pounds per acre of stream bottom.

*Many of the terms appearing here were taken from "Glossary of Commonly Used Biological and Related Terms in Water and Waste Water Control," by Jack R. Geckler, Kenneth M. Mackenthun, and William Marcus Ingram, Public Health Service Publication No. 999-WP-2, pp. i-22, 1963. (Note: Many terms have been added to those which appeared in this cited publication.)

ANNELIDS—Segmented worms, as distinguished from the non-segmented roundworms and flatworms. Most are marine; however, many live in soil or fresh water. Aquatic forms may establish dense populations in the presence of rich organic deposits. Common examples of segmented worms are earthworms, sludgeworms, sandworms, and leeches.

AQUATIC SOW BUGS (Isopoda)—Macroscopic aquatic crustaceans that are flat from top to bottom. Most are marine and estuarine. They are scavengers that live secretively under rocks and among vegetation and debris.

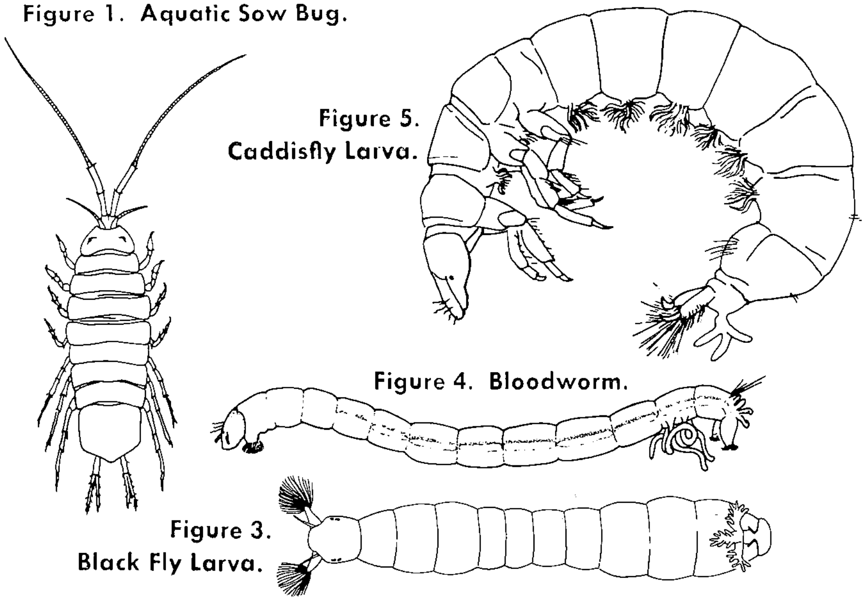


Figure 1. Aquatic Sow Bug.

Figure 5. Caddisfly Larva.

Figure 4. Bloodworm.

Figure 3. Black Fly Larva.

ARMORED FLAGELLATES—Flagellates having a cell wall composed of distinct, tightly arranged plates. The wall is usually thick and rough.

ARTIFICIAL SUBSTRATE—A device placed in the water for a period extending to a few weeks that provides living spaces for a multiplicity of drifting and natural-born organisms that would not otherwise be at the particular spot because of limiting physical habitat. Examples of artificial substrates include glass slides, tiles, bricks, wooden shingles, concrete blocks, multiplate-plate samplers, and brush boxes.

ACUTE TOXICITY—Toxicity that is fast-acting in its ability to produce death.

ADAPTATION—A change in the structure, form, or habit of an organism resulting from a change in its environment.

AEROBIC ORGANISM—An organism that thrives in the presence of oxygen.

ALGAE (Alga)—Simple plants, many microscopic, containing chlorophyll. Most algae are aquatic and may produce a nuisance when environmental conditions are suitable for prolific growth.

ALGICIDE—A specific chemical highly toxic to algae. Algicides are often applied to water to control nuisance algal blooms.

ALGOLOGY—The study of algae.

ALKYL BENZENE SULFONATE (ABS)—Most household detergents and commercial and industrial cleansers contain the anionic surface-active agent, ABS. As ABS is not found in natural substances, its presence in water is evidence of contamination by sewage or other man-made wastes.

ALLUVIAL FANS—A fan-shaped deposit of silt, sand, gravel or other fine materials from a stream where its gradient lessens abruptly as in the discharge of a stream into a lake or a river into an ocean.

AMPHIBIOUS ORGANISM—An organism adapted for life on land or in water.

AMPHIPODS (See Scuds)

ANADROMOUS FISHES—Fishes that spend a part of their life in the sea or lakes, but ascend rivers at more or less regular intervals to spawn. Examples are sturgeon, shad, salmon, trout, and striped bass.

ANAEROBIC ORGANISM—A microorganism that thrives best, or only, when deprived of oxygen.

ANEMOMETER—An instrument for measuring the force or velocity of the wind.

ASSIMILATION—The transformation of absorbed nutrients into body substances.

ASSOCIATION—An association in biology includes the entire organism population of a given habitat with two or more organism species dominating the group.

AUFWUCHS—Those organisms that attach firmly to a substrate but do not penetrate it (in contrast to plants rooted in the bottom or certain parasites). Aufwuchs comprise all attached organisms except the macrophytes in contrast to the more restricted English equivalent "periphyton" which includes the plants and animals adhering to parts of rooted aquatic plants.

AUTOTROPHIC—Self-nourishing; denoting the green plants and those forms of bacteria that do not require organic carbon or nitrogen, but can form their own food out of inorganic salts and carbon dioxide.

AUTOTROPHIC ORGANISM—An organism capable of constructing organic matter from inorganic substances.

BATHYTHERMOGRAPH—A device for recording the temperature at various depths in the oceans. As the instrument is lowered into the water the instrument plots the temperature and the pressure at various depths.

BENTHIC REGION—The bottom of all waters; the substratum that supports the benthos.

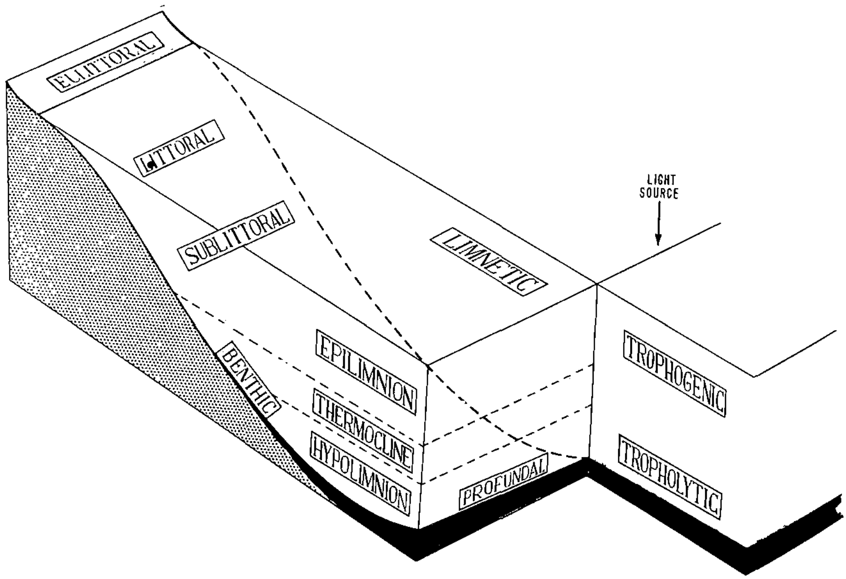



Figure 2. Lake Zones and Regions.

BENTHOS—Aquatic bottom-dwelling organisms. These include: (1) sessile animals, such as the sponges, barnacles, mussels, oysters, some of the worms, and many attached algae; (2) creeping forms, such as snails and flatworms; and (3) burrowing forms, which include most clams and worms.

BIO-ASSAY—A determination of the concentration of a given material by comparison with a standard preparation; or the determination of the quantity necessary to affect a test animal under stated laboratory conditions.

BIOMASS—The weight of all life in a specified unit of environment, for example, a square foot of stream bottom. An expression dealing with the total mass or weight of a given population, both plant and animal.

BIOTA—All living organisms of a region.

BIVALVE—An animal with a hinged two-valve shell; examples are the clam and oyster.

BLACK FLY LARVAE (Simuliidae)—Aquatic larvae that produce a silk-like thread with which they anchor themselves to objects in swift waters. With a pair of fan-shaped structures, a larva of this type produces a current of water toward its mouth and from this water ingests smaller organisms. The adults are terrestrial; females feed on the blood of higher animals.

BLOOD GILLS—Delicate blood-filled sacs that are found in certain insects. They are a taxonomic characteristic and are found near the posterior on the ventral surface of midge larvae. Most midge larvae with ventral blood gills are associated with organically enriched stream beds.

BLOODWORMS (Tendipedidae = Chironomidae)—Cylindrical elongated midge larvae with pairs of prolegs on both the first thoracic and last abdominal segments. Although many species are blood-red in color, some are pale yellowish, yellowish red, brownish, pale greenish yellow, and green. Most feed on diatoms, algae, tissues of aquatic plants, decaying organic matter, and plankton. Some are associated with rich organic deposits. Midge larvae are important as food for fishes.

BLOOM—A readily visible concentrated growth or aggregation of plankton (plant and animal).

BLUE-GREEN ALGAE—A group of algae with a blue pigment, in addition to the green chlorophyll. A stench is often associated with the decomposition of dense blooms of blue-green algae in fertile lakes.

BOTULISM—Poisoning by the toxin of a bacillus. It may be found in imperfectly canned foods.

BRUSH BOX (See Artificial Substrate)

CADDISFLY LARVAE (Trichoptera)—Aquatic larvae found in a variety of habitats. Many build cases of small rocks, shells, wood, and plants and feed upon plant tissue and small animals captured in nets they place near the case entrance. Adults have well-developed wings but no functional mouth parts. Eggs are deposited on sticks or stones in water.

CATADROMOUS FISHES—Fishes that feed and grow in fresh water, but return to the sea to spawn. The best-known example is the American eel.

CENTRARCHIDAE (See Sunfish)

CERCARIAE—The tailed, immature stage of a parasitic flatworm.

CHARA—A family of algae possessing cylindrical whorled branches. The plants grow only in highly alkaline water, from the bottom, and usually have a coating of lime that can be felt between the fingers. Chara should not be confused with submerged higher aquatic plants.

CHIRONOMIDAE (See Bloodworms)

CHLOROPHYLL—The green coloring matter in plants, partly responsible for photosynthesis.

CLADOCERA—A group of small, chiefly fresh-water, crustaceans, often known as water fleas.

CLEAN WATER ASSOCIATION—An association of organisms, usually characterized by many different kinds (species). These associations occur in natural unpolluted environments. Because of competition, predation, etc., however, relatively few individuals represent any particular species.

COARSE OR ROUGH FISH—Those species of fish considered to be of poor fighting quality when taken on tackle, and of poor food quality. These fish may be undesirable in a given situation, but

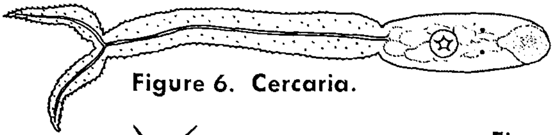


Figure 6. Cercaria.

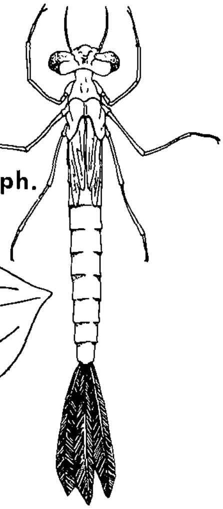


Figure 7. Damselfly Nymph.

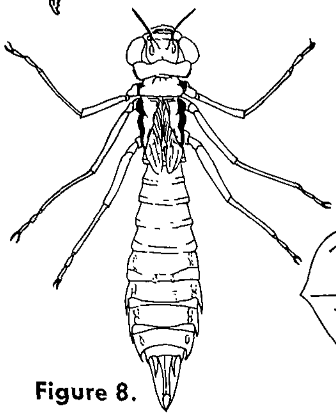


Figure 8. Dragonfly Nymph.

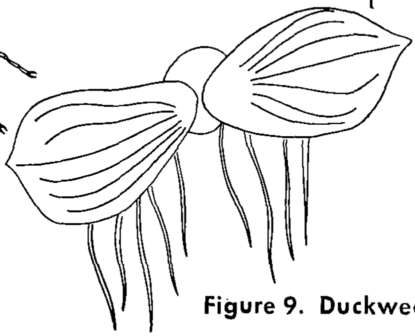


Figure 9. Duckweed.

at times may be classified differently, depending upon their usefulness. Examples include carp, goldfish, gar, suckers, bowfin, gizzard shad, goldeneye, mooneye, and certain kinds of catfish.

COELENTERATE—A group of aquatic animals that have gelatinous bodies, tentacles, and stinging cells. These animals occur in great variety and abundance in the sea and are represented in fresh water by a few types. Examples are hydra, corals, sea-anemones, and jellyfish.

COLD-BLOODED ANIMALS—Animals that lack a temperature-regulating mechanism that offsets external temperature changes. Their temperature fluctuates to a large degree with that of their environment. Examples are fish, shellfish, and aquatic insects.

CONSUMERS—Organisms that consume solid particles of organic food material. Protozoa are examples of consumer organisms.

CORYDALIDAE (See Hellgrammites)

CRUSTACEA—Mostly aquatic animals with rigid outer coverings, jointed appendages, and gills. Examples are crayfish, crabs, barnacles, water fleas, and sow bugs.

CURRENT, EBB—(See Ebb Current)

CUTICULAR PLATE—A hard chitinous or calcareous plate on the skin.

DAMSELFLY NYMPH (Odonata)—The immature damselfly. This aquatic insect nymph has an enormous grasping lower jaw and three flat leaf-like gill plates that project from the posterior of the abdomen. Nymphs live most of their lives searching for food among submerged plants in still water; a few cling to plants near the current's edge; and a very few cling to rocks in flowing water. The carnivorous adults capture lesser insects on the wing.

DAPHNIA—(See Water Fleas)

DEGRADATION ZONE—As organic pollution enters a stream, a zone of degradation is established. The BOD is increased and the dissolved oxygen is decreased. Sludge begins to accumulate, turbidity increases. Sewage molds, fungi, and filamentous bacteria may occur in abundance. The general bacterial population is increased and the intolerant or sensitive bottom-dwelling organisms are eliminated. Sludgeworms, rat-tailed maggots, some very tolerant midge larvae (usually equipped with ventral blood gills), and occasionally one or two species of leeches are found in small numbers.

DELTA—An alluvial deposit at the mouth of a river.

DERMATITIS—Any inflammation of the skin. One type may be caused by the penetration beneath the skin of a cercaria found in water; this form of dermatitis is commonly called "swimmer's itch."

DIATOMETER—An apparatus that holds microscopic slides in the water. It is held in place by means of floats and an anchor. Living diatoms, by means of their thin gelatinous coating, become attached to the glass slides. The slides are removed from the diatometer, at intervals generally of 14 days, dried, and shipped to the laboratory for study, identification, and enumeration.

DIATOMS—Organisms closely associated with algae that are characterized by the presence of silica in the cell walls which are sculptured with striae and other markings, and by the presence of a brown pigment associated with the chlorophyll.

DRAGONFLY NYMPH (Odonata)—The immature dragonfly. This aquatic insect nymph has gills on the inner walls of its rectal respiratory chamber. It has an enormous grasping lower jaw that it can extend forward to a distance several times the length of its head. Although many of these nymphs climb among aquatic plants, most sprawl in the mud where they lie in ambush to await their prey. The carnivorous adults capture lesser insects on the wing.

DUCKWEED—A free-floating aquatic flowering plant possessing fronds resembling tiny green leaves. Small roots beneath the leaves easily distinguish this plant from algae.

DYSTROPHIC LAKES—Brown-water lakes with a very low lime content and a very high humus content. These lakes often lack nutrients.

EBB CURRENT—The movement of the tidal current away from shore or down a tidal stream.

EBB TIDE—A nontechnical term referring to that period of tide between a high water and the succeeding low water; falling tide.

EBULLITION—The state of boiling or bubbling up, as in the emission of gas from an actively decomposing sludge deposit.

ECOLOGY—The branch of biology that deals with the interrelationships of living organisms and their environments, and to each other.

ECOSYSTEM—An ecological system; the interaction of living organisms and the nonliving environment producing an exchange of materials between the living and the nonliving.

EDDY CURRENT—A circular movement of water of comparatively limited area formed on the side of a main current. Eddies may be created at points where the main stream passes projecting obstructions.

EKMAN DREDGE—The standard spring-loaded device used for sampling soft bottoms. The body of the dredge consists of a square box of sheet brass (6 by 6, 9 by 9, or 12 by 12 inches). The lower opening of this box is closed by a pair of strong brass jaws that snap shut when the springs are released. When the jaws are fully pulled apart, the bottom of the dredge is open.

EMERGENT AQUATIC PLANTS—Plants that are rooted at the bottom but project above the water surface. Examples are cattails and bulrushes.

ENTOMOLOGIST—A specialist in the study of insects.

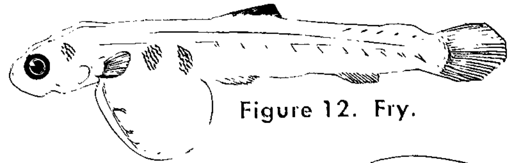


Figure 12. Fry.

Figure 10. Ekman Dredge.

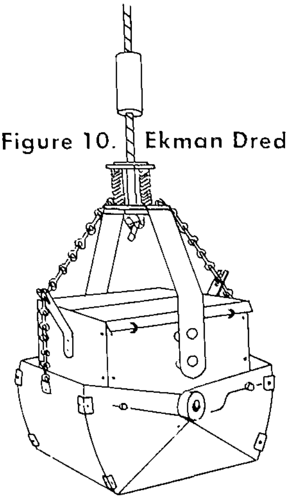
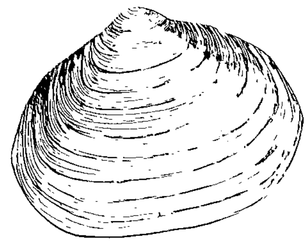


Figure 11.
Fingernail Clam.



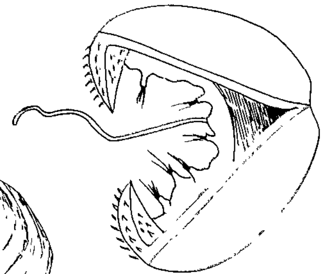


Figure 13.
Glochidium.

ENVIRONMENT—The sum of all external influences and conditions affecting the life and the development of an organism.

EPHEMERIDAE—(See Mayfly Naiads)

EPILIMNION—That region of a body of water that extends from the surface to the thermocline and does not have a permanent temperature stratification. (See Figure 2)

EPITHELIAL LAYER—A cellular tissue covering all free body surfaces.

ERISTALIS—(See Rat-Tailed Maggot)

ESTUARY—That portion of a stream influenced by the tide of the body of water into which it flows or a bay, at the mouth of a river, where the tide meets the river current.

EULITTORAL ZONE—The shore zone of a body of water between the limits of water-level fluctuation. (See Figure 2)

EUPHOTIC ZONE—The lighted region that extends vertically from the surface to the level at which photosynthesis fails to occur because of ineffective light penetration.

EURYHALINE—Organisms that are able to live in waters of a wide range of salinity.

EURYTOPIC ORGANISMS—Organisms with a wide range of tolerance to a particular environmental factor. Examples are sludgeworms and bloodworms.

EUTROPHICATION—The intentional or unintentional enrichment of water.

FACULTATIVE AEROBE—An organism that although fundamentally an anaerobe can grow in the presence of free oxygen.

FACULTATIVE ANAEROBE—An organism that although fundamentally an aerobe can grow in the absence of free oxygen.

FALL OVERTURN—A physical phenomenon that may take place in a body of water during the early autumn. The sequence of events leading to fall overturn include: (1) cooling of surface waters, (2) density change in surface waters producing convection currents from top to bottom, (3) circulation of the total water volume by wind action, and (4) vertical temperature equality, 4°C. The overturn results in a uniformity of the physical and chemical properties of the water.

FATHOM—A unit of measurement equal to 6 feet (1.83 meters).

FAUNA—The entire animal life of a region.

FINGERNAIL CLAMS (Sphaeriidae)—Small clams, usually less than one-half inch in diameter, that give live birth to shelled young.

FLATWORMS (Platyhelminthes)—Nonsegmented worms, flattened from top to bottom. In all but a few of the flatworms complete male and female reproductive systems are present in each individual. Most flatworms are found in water, moist earth, or as parasites in plants and animals.

FLOATING AQUATIC PLANTS—Plants that wholly or in part float on the surface of the water. Examples are water lilies, water shields, and duckweeds.

FLOC—A small, light, loose mass, as of a fine precipitate.

FLOCCULENT—Reassembling tufts of cotton or wool; denoting a fluid containing numerous shreds of fluffy, gray-white particles; containing or consisting of flocs.

FLOOD CURRENT—The movement of the tidal current toward the shore or up a tidal stream.

FLOOD TIDE—A nontechnical term referring to that period of tide between low water and the succeeding high water; a rising tide.

FLORA—The entire plant life of a region.

FOOD-CHAIN—The dependence of organisms upon others in a series for food. The chain begins with plants or scavenging organisms and ends with the largest carnivores.

FOOD-CYCLE—All the interconnecting food-chains in a community.

FRY (Sac Fry)—The stage in the life of a fish between the hatching of the egg and the absorption of the yolk sac. From this stage until they attain a length of 1 inch the young fish are considered advanced fry.

FUNGI (Fungus)—Simple or complex organisms without chlorophyll. The simpler forms are one-celled; the higher forms have branched filaments and complicated life cycles. Examples of fungi are molds, yeasts, and mushrooms.

FUNGICIDE—Substances or a mixture of substances intended to prevent, destroy, or mitigate any fungi.

GAME FISH—Those species of fish considered to possess sporting qualities on fishing tackle. These fish may be classified as undesirable, depending upon their usefulness. Examples of fresh-water game fish are salmon, trout, grayling, black bass, muskellunge, walleye, northern pike, and lake trout.

GELATINOUS MATRIX—Jelly-like intercellular substance of a tissue; a semisolid material surrounding the cell wall of some algae.

GLOBULAR—Having a spherical shape; globe-shaped.

GLOCHIDIUM—The larvae of fresh-water mussels. These larvae are temporary parasites that live on the gills, fins, and general body surface of many fish.

GREEN ALGAE—Algae that have pigments similar in color to those of higher green plants. Common forms produce algal mats or floating "moss" in lakes.

HALOPHYTE—Plants capable of thriving on salt-impregnated soils.

HELLGRAMMITES (Corydalidae)—Dobsonfly larvae. Full-grown larvae are 2 to 3 inches in length; they have a dark-brown rough-looking skin, large jaws, and posterior hooks. The aquatic larval stage lasts 2 to 3 years. They are secretive and predaceous, living under rocks and debris in flowing water. These larvae are considered one of the finest live baits by fishermen. Pupation occurs on shore, under rocks and debris near the stream edge. The terrestrial adults are short lived.

HERBICIDE—Substances or a mixture of substances intended to control or destroy any vegetation.

HERBIVORE—An organism that feeds on vegetation.

HETEROCYST—A specialized vegetative cell in certain filamentous blue-green algae; larger, clearer, and thicker-walled than the regular vegetative cells.

HETEROTROPHIC ORGANISMS—Organisms that are dependent on organic matter for food.

HIGHER AQUATIC PLANTS—Flowering aquatic vascular plants. (These are separately categorized herein as Emergent, Floating, and Submerged Aquatic Plants.)

HIGHER HIGH WATER (HHW)—The higher of the two high waters of any tidal day. The single high water occurring daily during periods when the tide is diurnal is considered to be a higher high water.

HIGHER LOW WATER (HLW)—The higher of two low waters of any tidal day.

HIGH TIDE; HIGH WATER (HW)—The maximum height reached by each rising tide.

HIRUDIN—A substance extracted from salivary glands of leeches that prevents coagulation of the blood.

HIRUDINEA (See Leeches)

HOLOMICTIC LAKES—Lakes that are completely circulated to the bottom at time of winter cooling.

HOMOIOTHERMIC ANIMALS (WARM-BLOODED ANIMALS)—Animals that possess a temperature-regulating mechanism to maintain a more or less constant body temperature.

HOMOTHERMOUS—Having the same temperature throughout.

HYDROPHOTOMETER—A submersible device to measure the intensity of illumination flux beneath the surface in a body of water.

HYPOLIMNION—The region of a body of water that extends from the thermocline to the bottom of the lake and is removed from surface influence. (See Figure 2)

ICTHYOLOGIST—A specialist in the study of fishes.

INDICATOR ORGANISMS—An organism, species, or community that indicates the presence of a certain environmental condition or conditions; the species composition and relative abundance of individual components of the bottom organism population are often used to define pollution by organic wastes.

INSECTICIDE—Substances or a mixture of substances intended to prevent, destroy, or repel insects.

INTOLERANT ORGANISM—Organisms that are sensitive to pollution, especially organic pollution, and are either killed or driven out of the area when the environment is fouled.

INVERTEBRATES—Animals without backbones.

ISOPODA—(See Aquatic Sow Bugs)

KEMMERER WATER SAMPLER—An instrument designed to collect a known volume of water from a predetermined depth. The sampler construction essentially consists of a brass cylinder with closable rubber stoppers on each end. It is suspended in the water with a rope; closure is accomplished when a brass messenger, which is sent down the rope, strikes a tripping device.

LAKE AND RESERVOIR TURNOVER—In deep lakes, the seasons induce a cycle of physical and chemical changes in the water that are often conditioned by temperature. For a few weeks in the spring, and again in the autumn, water temperatures may be homogeneous from the top of a water body to the bottom. Vertical water density is also homogeneous, and it becomes possible for the wind to mix the water in a lake, distributing nutrients and flocculent bottom solids from the deeper waters. This is a period of water turnover.

LARVA—The wormlike form of an insect on issuing from the egg.

$LD_{50}$—(See Median Lethal Dose)

LEECHES (Hirudinea)—Segmented worms, flat from top to bottom, with terminal suckers that are used for attachment and locomotion. Various species may be parasites, predators, or scavengers; most are aquatic.

LENITIC OR LENTIC ENVIRONMENT—Standing water and its various intergrades. Examples of lenitic environments are lakes, ponds, and swamps.

LIFE CYCLE—The series of stages in the form and mode of life of an organism, i.e., the stages between successive recurrences of a certain primary stage such as the spore, fertilized egg, seed, or resting cell.

LIMNETIC ZONE—The open-water region of a lake. This region

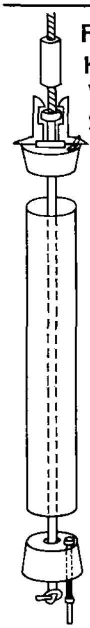


Figure 15. Kemmerer Water Sampler.

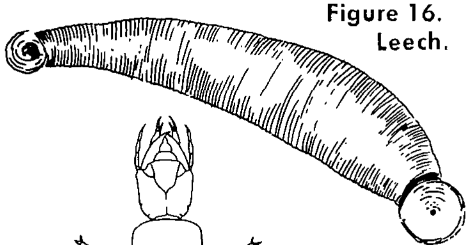


Figure 16. Leech.

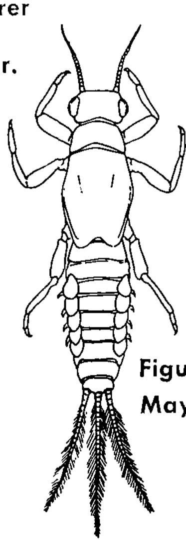


Figure 17. Mayfly Naiad.

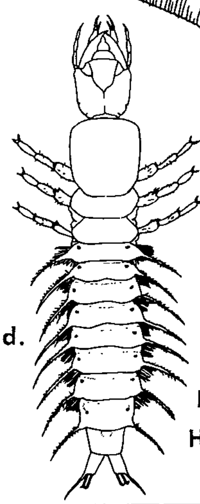


Figure 14. Hellgrammite.

supports plankton and fish as the principal plants and animals. (See Figure 2)

LIMNOLOGY—The study of the physical, chemical, and biological aspects of inland waters.

LITTORAL ZONE—The shoreward region of a body of water. (See Figure 2)

LOTIC ENVIRONMENT—Running waters, such as streams or rivers.

LOWER HIGH WATER (LHW)—The lower of the two high waters of any tidal day.

LOWER LOW WATER (LLW)—The lower of the two waters of any tidal day. The single low water occurring daily during periods when the tide is diurnal is considered to be a lower low water.

LOW FLOW AUGMENTATION—Increasing of an existing flow. The total flow of a stream can seldom be increased but its ability to assimilate waste can generally be improved by storage of floodflows and their subsequent release when natural flows are low and water quality conditions are poor.

LUMEN—The space in the interior of a tubular structure such as an artery or the intestine.

LYSIMETER—A device for measuring percolation of water through soils and determining the soluble constituents removed in drainage.

MACROORGANISMS—Plant, animal, or fungal organisms visible to the unaided eye.

MAYFLY NAIADS (Ephemeridae)—The immature mayfly. Paired gills are attached to the upper surface of the outer edge of some or all of the first seven abdominal segments. The abdomen terminates in three, rarely two, slender tails. Mouth parts are particularly suited for raking diatoms and rasping decaying plant stems. The terrestrial adults lack functional mouth parts and live only a few hours.

MEAN HIGHER HIGH WATER (MHHW)—The average height of the higher high waters over a 19-year period. For shorter periods of observation, corrections are applied to eliminate known variations and reduce the result to the equivalent of a mean 19-year value.

MEAN HIGH WATER (MHW)—The average height of the high waters over a 19-year period. For shorter periods of observations, corrections are applied to eliminate known variations and reduce the result to the equivalent of a mean 19-year value. All high water heights are included in the average where the type of tide is either semidiurnal or mixed. Only the higher high water heights are included in the average where the type of tide is diurnal. So determined, mean high water in the latter case is the same as mean higher high water.

MEAN LOWER LOW WATER (MLLW)—Frequently abbreviated lower low water. The average height of the lower low waters over a 19-year period. For shorter periods of observations, corrections are applied to eliminate known variations and reduce the result to the equivalent of a mean 19-year value.

MEAN LOW WATER (MLW)—The average height of the low waters over a 19-year period. For shorter periods of observations, corrections are applied to eliminate known variations and reduce the result to the equivalent of a mean 19-year value. All low water heights are included in the average where the type of tide is either semidiurnal or mixed. Only the lower low water heights are included in the average where the type of tide is diurnal. So determined, mean low water in the latter case is the same as mean lower low water.

MEDIAN LETHAL DOSE ($LD_{50}$)—The dose lethal to 50 percent of a group of test organisms for a specified period. The dose material may be ingested or injected.

MEDIAN TOLERANCE LIMIT ($TL_m$)—The concentration of the tested material in a suitable diluent (experimental water) at which just 50 percent of the test animals are able to survive for a specified period of exposure.

MEROMICTIC LAKES—Lakes in which dissolved substances create a gradient of density differences in depth, preventing complete mixing or circulation of the water.

MESENTERIC VEIN—The large vein leading from the intestines in the abdominal cavity.

MICROORGANISM—Any minute organism invisible or barely visible to the unaided eye.

MINNOWS (Cyprinidae)—The family of fishes including such forms as shiners, dace, and carp.

MIRACIDIUM—The ciliated free-swimming larva of a trematode worm.

MOLLUSCICIDE—Substances or a mixture of substances intended to destroy or control snails. Copper is commonly used.

MOLLUSK (Mollusca)—A large animal group including those forms popularly called shellfish (but not including crustaceans). All have a soft unsegmented body protected in most instances by a calcareous shell. Examples are snails, mussels, clams, and oysters.

MORPHOMETRY—The physical shape and form of a water body.

MOSS—Any bryophytic plant characterized by small, leafy, often tufted stems bearing sex organs at the tips.

MOTILE—Exhibiting or capable of spontaneous movement.

MUSSEL POISON—(See Shellfish Poison)

MYCOLOGY—The study of fungi.

NAIAD—The immature instar or developmental form that is characteristic of the preadult stage in insects with incomplete metamorphosis. Examples include stoneflies, mayflies, and dragon- and damselflies.

NANOPLANKTON—Very small plankton not retained by a plankton net equipped with No. 25 silk bolting cloth.

NEKTON—Swimming organisms able to navigate at will.

NEMATODA—Unsegmented roundworms or threadworms. Some are free living in soil, fresh water, and salt water; some are found living in plant tissue; others live in animal tissue as parasites.

NEUSTON—Organisms resting or swimming on the surface film of the water.

NYMPH—An immature developmental form that is characteristic of the preadult stage in insects with gradual metamorphosis.

OCEANOGRAPHY—The study of the physical, chemical, geological, and biological aspects of the sea.

OCULAR MICROMETER—A scaled glass disc that is used in making microscopic measurements. It is fitted on the diaphragm of a microscope ocular.

OLIGOTROPHIC WATERS—Waters with a small supply of nutrients; thus, they support little organic production.

ORANGE PEEL BUCKET—A dredge consisting of four sectors designed to take a hemispherical bite out of the bottom. The sectors are operated by a large wheel-and sprocket mechanism within the upper framework. Usually a canvas sleeve is placed over the upper works to prevent washing out of the contents when the bucket is being hauled up.

ORGANIC DETRITUS—The particulate remains of disintegrated plants and animals.

OSTRACODS—Small (just visible to the unaided eye), active, mostly fresh water, organisms having the body enclosed in a bivalve shell composed of right and left valves.

OXYGEN-DEBT—A phenomenon that occurs in an organism when available oxygen is inadequate to supply the respiratory demand. During such a period the metabolic processes result in the accumulation of breakdown products that are not oxidized until sufficient oxygen becomes available.

PAPILLA—Any small nipplelike process.

PARASITE—An organism that lives on or in a host organism from which it obtains nourishment at the expense of the latter during all or part of its existence.

PEAKING—The use of hydropower to meet either maximum or rapid changes in power demands.

PEARL BUTTON CLAMS (Unionidae)—Large fresh-water clams. The shell has a thick mother-of-pearl layer. The thick-shelled members of this family are utilized in the manufacturing of buttons.

PELAGIC ZONE—The free-water region of a sea. (Pelagic refers to the sea, limnetic refers to bodies of fresh water.)

PENSTOCK—A sluice for regulating flow of water, a conduit for conducting water.

PERIPHYTON—The association of aquatic organisms attached or clinging to stems and leaves of rooted plants or other surfaces projecting above the bottom.

PESTICIDE—Any substance used to kill pest organisms including insecticides, herbicides, algicides, fungicides, and bacteriacides.

PETERSEN DREDGE—A sturdy steel or iron clam-type dredge widely used for taking samples from hard bottoms, such as sand, gravel, marl, clay, and similar materials. It is so constructed that, both by its own weight and by the leverage exerted by its closing mechanism, it bites its way into hard bottoms deep enough to secure a satisfactory sample. The area sampled varies from 0.6 to 0.9 square foot, depending on individual dredge construction.

PHOTIC ZONE—The surface waters that are penetrated by sunlight.

PHOTOSYNTHESIS—The process by which simple sugars are manufactured from carbon dioxide and water by living plant cells with the aid of chlorophyll in the presence of light.

PHOTOTROPISM—Movement in response to a light gradient; for example, a movement towards light is positive phototropism.

PHOTOMETER—An instrument used to measure the intensity of light in water.

PHYTOPLANKTON—Plant microorganisms, such as certain algae, living unattached in the water.

PISCICIDE—Substances or a mixture of substances intended to destroy or control fish populations.

PLANKTON—Plant and animal organisms of small size, mostly microscopic, that either have relatively small powers of locomotion or drift in the water subject to the action of waves and currents.

PLANKTON NET—A cloth net, usually coneshaped, used to collect plankton. Plankters separated from water by means of a net are generally referred to as net plankton and represent only a fraction of the total population. Silk bolting cloth is regarded as the best material for plankton nets.

PLASTIDS—A body in a plant cell that contains photosynthetic pigments.

PLATYHELMENTHES—(See Flatworms)

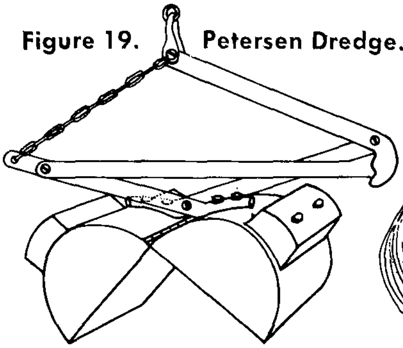


Figure 19. Petersen Dredge.

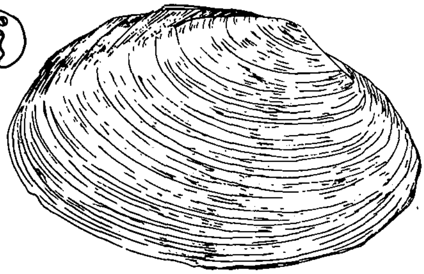


Figure 18. Pearl Button Clam.

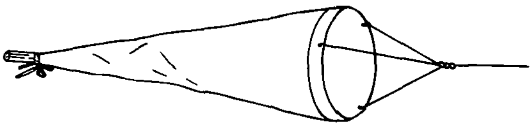


Figure 20. Plankton Net.

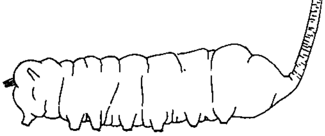


Figure 21. Rat-Tailed Maggot.

PLECOPTERA—(See Stonefly Nymphs)

POIKILOTHERMIC ANIMALS (See Cold-Blooded Animals)

POOL ZONE—The deep-water area of a stream, where the velocity of current is reduced. The reduced velocity provides a favorable habitat for plankton. Silts and other loose materials that settle to the bottom of this zone are favorable for burrowing forms of benthos.

PORIFERA—(See Sponges)

POTAMOLOGY—The study of the physical, chemical, geological, and biological aspects of rivers.

PRIMARY PRODUCTIVITY—The rate of photosynthetic carbon fixation by plants and bacteria forming the base of the food chain.

PRODUCERS—Organisms that synthesize their own organic substance from inorganic substances; for example, plants.

PRODUCTION (Productivity)—A time-rate unit of the total amount of organisms grown.

PROFUNDAL ZONE—The deep- and bottom-water area beyond the depth of effective light penetration. All of the lake floor beneath the hypolimnion. (See Figure 2)

PROTOZOA—Organisms consisting either of a single cell or of aggregates of cells, each of which performs all the essential functions in life. They are mostly microscopic in size and largely aquatic.

PROTOZOOLOGIST—A specialist in the study of protozoa.

PSYCHODA—(See Sewage Fly Larvae.)

PUPA—An intermediate, usually quiescent, form following the larval stage in insects, and maintained until the beginning of the adult stage.

PYRHELIOMETER—An instrument for measuring the rate at which heat energy is received from the sun (usually expressed as gm Cal./$cm^2$/minute).

RAPIDS ZONE—The shallow-water area of a stream, where velocity of current is great enough to keep the bottom clear of silt and other loose materials, thus providing a firm bottom. This zone is occupied largely by specialized benthic or periphytic organisms that are firmly attached to or cling to a firm substrate.

RAT-TAILED MAGGOT (Tubifera = Eristalis)—An aquatic fly maggot usually found in foul, often septic, water. It possesses a three-segmented, telescopic air tube that extends through the water surface, enabling the maggot to breathe from the atmosphere. The larvae live on decayed organic material.

RECOVERY ZONE—Following the zone of active decomposition, a zone of stream recovery may extend for miles. In this zone, the BOD decreases and the dissolved oxygen increases to the unpolluted concentration. Molds and fungi have been replaced by a growth of algae. Rotifers and crustacea succeed the ciliates. The population abundance decreases and the number of species represented within the bottom community increases. Sowbugs and fingernail clams may be very abundant. Several species of snails, leeches, midge larvae, and other fly larvae are also numerous. Intolerant or sensitive bottom-dwelling forms such as stoneflies, mayflies, and caddisflies may appear near the end of the zone.

REDD—A type of fish-spawning area associated with running water and clean gravel. Fish moving upstream sequentially dig a pocket, deposit and fertilize eggs, and then cover the spawn with gravel from the next upstream pocket. Fishes that utilize this type of spawning area include some trouts, salmons, and minnows.

REDIA—A larval stage of certain (flatworm) trematoda.

RED TIDE—A visible red-to-orange coloration of an area of the sea caused by the presence of a bloom of certain "armored" flagellates.

REDUCERS—Organisms that digest food outside the cell wall by means of enzymes secreted for this purpose. Soluble food is then absorbed into the cell and reduced to a mineral condition. Examples are fungi, bacteria, protozoa, and nonpigmented algae.

RHEOTROPISM—Movement in response to the stimulus of a current gradient in water.

RIFFLE—A section of a stream in which the water is usually shallower and the current of greater velocity than in the connecting pools; a riffle is smaller than a rapid and shallower than a chute.

ROTIFERS (Rotatoria)—Microscopic aquatic animals, primarily free-living fresh-water forms that occur in a variety of habitats. Approximately 75 percent of the known species occur in the littoral zone of lakes and ponds. The more dense populations are associated with a substrate of submerged aquatic vegetation. Most forms ingest fine organic detritus for food, whereas others are predaceous.

SAC FRY—(See Fry)

SAPROBIENSYSTEM—A European system of classifying organisms according to their response to the organic pollution in slow moving streams.

*Alpha-Mesosaprobic Zone*—Area of active decomposition, partly aerobic, partly anaerobic, in a stream heavily polluted with organic wastes.

*Beta-Mesosaprobic Zone*—That reach of stream that is moderately polluted with organic wastes.

*Oligosaprobic Zone*—That reach of a stream that is slightly polluted with organic wastes and contains the mineralized products of self-purification from organic pollution, but with none of the organic pollutants remaining.

*Polysaprobic Zone*—That area of a grossly polluted stream which contains the complex organic wastes that are decomposing primarily by anaerobic processes.

SAPROPHYTE—Any organism living on dead or decaying organic matter.

SCAVENGER—An organism that feeds upon decomposing organic matter.

SCUDS (Amphipods)—Macroscopic aquatic crustaceans that are laterally compressed. Most are marine and estuarine. Dense populations are associated with aquatic vegetation. Great numbers are consumed by fish.

**Figure 22. Scud.**

SECCHI DISK—A circular metal plate, 20 centimeters in diameter, the upper surface of which is divided into 4 equal quadrants and so painted that 2 quadrants directly opposite each other are black and the intervening ones white.

SEDGWICK-RAFTER CONCENTRATION METHOD—A procedure for the quantitative determination of plankton in water by use of a special funnel, a certain grade of sand, and bolting-cloth discs.

SEDGWICK-RAFTER COUNTING CELL—A plankton-counting cell consisting of a brass or glass receptacle 50 by 20 by 1 millimeter sealed to a 1- by 3-inch glass microscope slide. A rectanglular cover glass large enough to cover the whole cell is required. The cell has a capacity of exactly 1 milliliter.

SEICHE—A periodic oscillation of a body of water whose period is determined by the resonant characteristics of the containing basin as controlled by the physical dimensions. These periods generally range from a few minutes to an hour or more. (Originally the term was applied only to lakes but now also to harbors, bays, oceans, etc.)

SESSILE ORGANISMS—Organisms that sit directly on a base without support, attached or merely resting unattached on a substrate.

SESTON—The living and nonliving bodies of plants or animals that float or swim in the water.

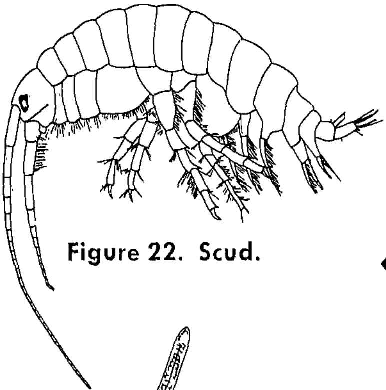


Figure 22. Scud.

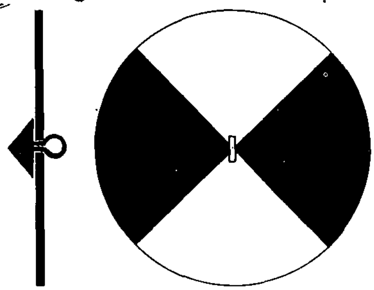


Figure 23. Secchi Disk.

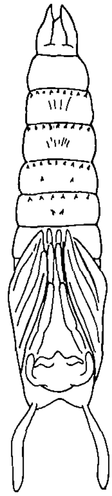


Figure 24. Sewage Fly Pupa.

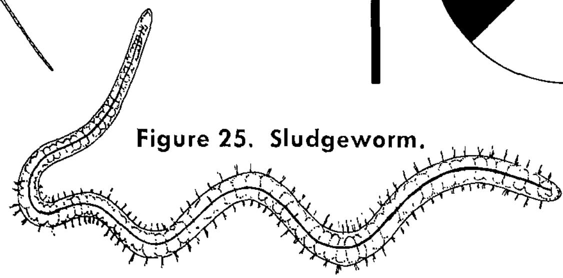


Figure 25. Sludgeworm.

SEWAGE FLY LARVAE (Psychoda)—A grayish-white, cylindrical larvae with hardened dorsal plates on the posterior segments. Larvae and pupae usually occur in filter beds of sewage treatment plants, in foul water, and in decaying organic matter. The terrestrial adults are small, less than 4 millimeters long, and moth-like, and often are a nuisance in areas near trickling-filter plants. The sewage fly has a 2-week life cycle.

SHELLFISH POISON (Mussel Poison)—A poison present in shellfish that have fed upon certain small marine phytoplankters in which the toxic principles exist. The shellfish concentrates the poison without harmful effects to itself, but man is poisoned through consumption of the toxic flesh.

SICKLE-SHAPED—Curved or crescent shaped.

SIMULIIDAE—(See Black Fly Larvae)

SLACK TIDE (Slack Water)—The state of a tidal current when its velocity is near zero, especially the moment when a reversing current changes direction and its velocity is zero. Sometimes considered the intermediate period between ebb and flood currents during which the velocity of the currents is less than 0.1 knot.

SLUDGEWORMS (Tubificidae)—Tolerant, aquatic, segmented worms that exhibit marked population increases in streams and rivers polluted with organic decomposable wastes.

SNAIL—An organism that typically possesses a coiled shell and crawls on a single muscular foot. Air-breathing snails, called pulmonates, do not have gills but obtain oxygen through a "lung" or pulmonary cavity. At variable intervals most pulmonate snails come to the surface of the water for a fresh supply of air. Gill-breathing snails possess an internal gill through which dissolved ogygen is removed from the surrounding water.

SPECIES (both singular and plural)—An organism or organisms forming a natural population or group of populations that transmit specific characteristics from parent to off-spring. They are reproductively isolated from other populations with which they might breed. Populations usually exhibit a loss of fertility when hybridizing.

SPHAERIIDAE—(See Fingernail Clams)

SPHAEROTILUS—A slime-producing, nonmotile, sheathed, filamentous, attached bacterium. Great masses are often broken from their "holdfasts" by currents and are carried floating downstream in gelatinous flocks.

SPONGES (Porifera)—One of the sessile animals that fasten to piers, pilings, shells, rocks, etc. Most live in the sea.

SPORE—A reproductive cell of a protozoan, fungus, or alga. In bacteria, spores are specialized resting cells.

SPRING OVERTURN—A physical phenomenon that may take place in a body of water during the early spring. The sequence of events leading to spring overturn include: (1) melting of ice cover, (2) warming of surface waters, (3) density change in surface waters producing convection currents from top to bottom, (4) circulation of the total water volume by wind action, and (5) vertical temperature equality, 4°C. The overturn results in a uniformity of the physical and chemical properties of the water.

STAGE MICROMETER—A standardized, accurately ruled scale, mounted on a glass slide. It is used to calibrate a microscope.

STANDING CROP—The biota present in an environment at a selected point in time.

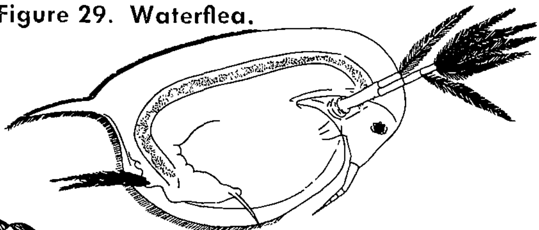


Figure 29. Waterflea.

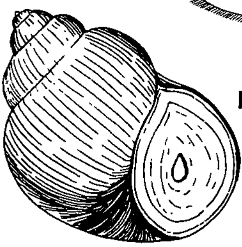


Figure 26. Snail.

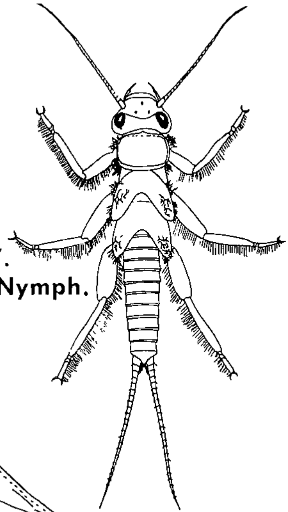


Figure 27. Stonefly Nymph.

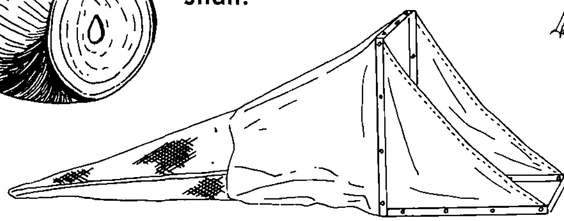


Figure 28. Surber Stream Bottom Sampler.

STENOTOPIC ORGANISMS—Organisms with a narrow range of tolerance for a particular environmental factor. Examples are trout, stonefly nymphs, etc.

STONEFLY NYMPHS (Plecoptera)—Immature stoneflies. The nymphs live approximately 1 year in the unpolluted, rapidly moving water required for their development. They live under rocks, in cracks of submerged logs, and in mats of debris. Most stonefly nypmhs are vegetarians; however, a number are predaceous and feed upon small insects and other aquatic invertebrates. The adults live only a few weeks; they are secretive creatures, resting on stones and sticks along the banks of streams.

SUBLITTORAL ZONE—The part of the shore from the lowest water level to the lower boundary of plant growth. (See Figure 2)

SUBMERGED AQUATIC PLANT—A plant that is growing or adapted to grow beneath the surface of the water. Examples are the pondweed and coontail.

SUNFISH (Centrarchidae)—Carnivorous fresh-water fish, all of which are spring spawners. The females utilize shallow depressions excavated by the males for nests; later the males guard the eggs and the young. Like other essentially carnivorous fish, the young feed first on microscopic organisms and later on invertebrates and vertebrates. Members of this family are generally divided into the following groups: (1) largemouth and smallmouth black bass, (2) crappies and the round sunfish, (3) true sunfish and rock bass, and (4) Sacramento perch of the Pacific Coast.

SURBER STREAM BOTTOM SAMPLER—A compact, lightweight, portable, quantitative bottom sampler especially suitable for sampling organisms from the stone or gravel bottoms of shallow streams possessing a strong current. Construction consists of two square metal frames of equal size hinged together. One frame carries a net of extra heavy bolting cloth; the other, when in working position, encloses the sampling area (1 square foot). Dislodged organisms are carried into the downstream net.

SURFACE AQUATIC PLANTS—Plants whose leaves float upon the surface of the water. Larger ones, such as the water lilies are rooted in the mud of the bottom, and bear great leaves that float upon the surface. The smaller ones such as duckweeds are free-floating.

SWIMBLADDER—An internal membranous gas-filled organ of many fishes. It may function as a hydrostatic or sense organ, or as part of the respiratory system.

SWIMMER'S ITCH—A rash produced on bathers by a parasitic flatworm in the cercarial stage of its life cycle. The organism is killed by the human body as soon as it penetrates the skin; however, the rash may persist for a period of about 2 weeks.

SYMBIOSIS—Two organisms of different species living together, one or both of which may benefit and neither is harmed.

SYNONOMY—A list of words of similar meaning; the scientific names (incorrect and correct), collectively, that have been used in different publications to designate a species or other group.

SYSTEMATICS—The science of organism classification.

TAILRACE—The channel into which the water from a water wheel or turbine is discharged.

TENDIPEDIDAE—(See Bloodworms.)

THERMOCLINE—The layer in a body of water in which the drop in temperature equals or exceeds 1 degree centigrade for each meter or approximately 3 feet of water depth.

TIDAL PRISM—The total amount of water that flows into the harbor or out again with movement of the tide, excluding any fresh water flow.

TIDE—The periodic rising and falling of the water that results from gravitational attraction of the moon and sun acting upon the rotating earth. Although the accompanying horizontal movement of the water resulting from the same cause is also sometimes called the tide, it is preferable to designate the latter as TIDAL CURRENT, reserving the name tide for the vertical movement.

$TL_m$ (See Median Tolerance Limit)

TOLERANT ASSOCIATION—An association of organisms capable of withstanding adverse conditions within the habitat. It is usually characterized by a reduction in species (from a clean water association), and an increase in individuals representing a particular species.

TOXICITY—Quality, state, or degree, of being toxic or poisonous.

TRACHEAL GILLS—Outgrowths of the skin, traversed by fine tracheal airtubes, that are common among insect larvae. The exchange of gases is between the water and the air contained within the tubes.

TRANSPIRATION—The loss of water in vapor form from a plant, mostly through plant pores.

TREMATODE—The common name for a parasitic worm of the class Trematoda, a fluke.

TRICHOPTERA—(See Caddisfly Larvae)

TROPHOGENIC REGION—The superficial layer of a lake in which organic production from mineral substances takes place on the basis of light energy. (See Figure 2)

TROPHOLYTIC REGION—The deep layer of a lake, where organic dissimilation predominates because of light deficiency. (See Figure 2)

TUBIFERA—(See Rat-Tailed Maggot)

TUBIFICIDAE—(See Sludgeworms)

TUBIFICIDS—(See Sludgeworms)

UNIONIDAE—(See Pearl Button Clams)

VENTRAL—Relating to the belly or the abdomen; opposed to dorsal.

VERTEBRATE—Animals with backbones.

WARM- AND COLD-WATER FISH—Warm-water fish include black bass, sunfish, catfish, gar, and others; whereas cold-water fish include salmon and trout, whitefish, miller's thumb, and blackfish. The temperature factor determining distribution is set by adaptation of the eggs to warm or cold water.

WATERFLEAS (Daphnia)—Mostly microscopic swimming crustaceans, often forming a major portion of the zooplankton population. The second antennae are very large and are used for swimming.

WHIPPLE OCULAR MICROMETER—A glass disc, marked with squares, that fits into a microscope ocular and is used to determine microscopic field areas for counting plankton.

WINTER KILL—The death of fishes resulting from unfavorable dissolved oxygen conditions under ice.

ZOOGLEA—Bacteria embedded in a jelly-like matrix formed as the result of metabolic activities.

ZOOPLANKTON—Animal microorganisms living unattached in water. They include small crustacea, such as daphnia and cyclops, and single-celled animals as protozoa, etc.

*Chapter III**

# GRAPHIC EXPRESSION OF BIOLOGICAL DATA IN WATER POLLUTION REPORTS

Many present day water pollution problems require attention to the responses of organisms exposed to the changing aquatic environment and fall within the sphere of interest of biologists. Frequently, the organisms of concern are desirable ones, such as fish and shellfish, which often respond to the pollution environment by becoming less numerous and deteriorating in quality. Of concern, also, are undesirable organisms, such as bloom-producing algae and slime growths, which sometimes become so numerous as to be a nuisance. In either situation, the consequences are costly—in one case, loss of a valuable resource—in the other, an expensive problem of organism control. In spite of these and still other areas of legitimate interest, the voice of the biologist too frequently has been feeble and unclear. There no doubt are a variety of causes to account for this. A vitally important one is the frequent failure of communications between biologists and others who share their interest and enthusiasm for water conservation. That failure of communication and possible remedies for it form the subject matter of this paper in the hope that the valuable biological work in this field will yield a more useful product.

Biological information pertinent to water pollution surveys has little value or utility unless presented in a form that is readily

*Taken From: "Graphic Expression of Biological Data in Water Pollution Reports," by W. M. Ingram and A. F. Bartsch, reprinted with permission from JOURNAL WATER POLLUTION CONTROL FEDERATION, VOL. 32, NO. 3, PP. 297-310 (MARCH 1960), WASHINGTON, D. C. 20016.

visualized and understood. Except for the median tolerance limit ($TL_m$), generally expressing satisfactorily the result of toxicity tests typically using fish, biological data pertinent in some way or other to stream conditions are often poorly presented for appraisal by the public and by persons not specifically trained in biological sciences. Once the funds have been expended to gather biological data, it is only reasonable in the interest of assuring maximum usefulness that they be presented in some clearcut form and described as succinctly as possible. It would be beneficial if biotic reflections of water quality could be expressed in meaningful numbers susceptible to mathematical manipulation as are BOD and some other data, but limited efforts in this direction have not yet been wholly successful. Accordingly, while it is possible for biotic data to show seriousness of pollution and need for remedial measures, such data do not indicate appropriate capacity or design of remedial works, and are not intended to do so.

In the past, and unfortunately at present also, it is not uncommon to find the biologist's report held aloof from that of his colleagues who addressed themselves concurrently to chemical, bacteriological, and engineering aspects of the same problem. Traditionally it appeared as an appendix, gave long lists of technical names of organisms found, and described the complexities of stream biology, but failed to relate biology clearly to the other data or to the essence of the problem. No thinking person will deny the importance of recording the names of organisms that make up the spectrum of life in various environments affected by pollution. Such information adds to the general store of ecological knowledge and has interest from a number of points of view. But too often such lists appear as an end in themselves rather than a data-assembling step preliminary to problem analysis.

Whether or not justified, it undoubtedly was the apparent sanctity of this type of thing that caused Wylie (1) to write: "Scientists—and especially biologists, whose disciplines have benefited less often than some others from an extreme need to communicate with congressmen and even the electorate—have for generations been free to immolate themselves in proud palaces of nomenclature. Today, a herpetologist can converse with an entomologist about science only up to the level of pregraduate study. Beyond that, interpreters are as indispensable as uncommon. Yet, instead of being embrarrassed [sic: embarrassed] by their terminological entombment they take pride in the matter. For many, indeed, facility in the local language represents their only palpable achievement after decades of learning and labor." The admonition im-

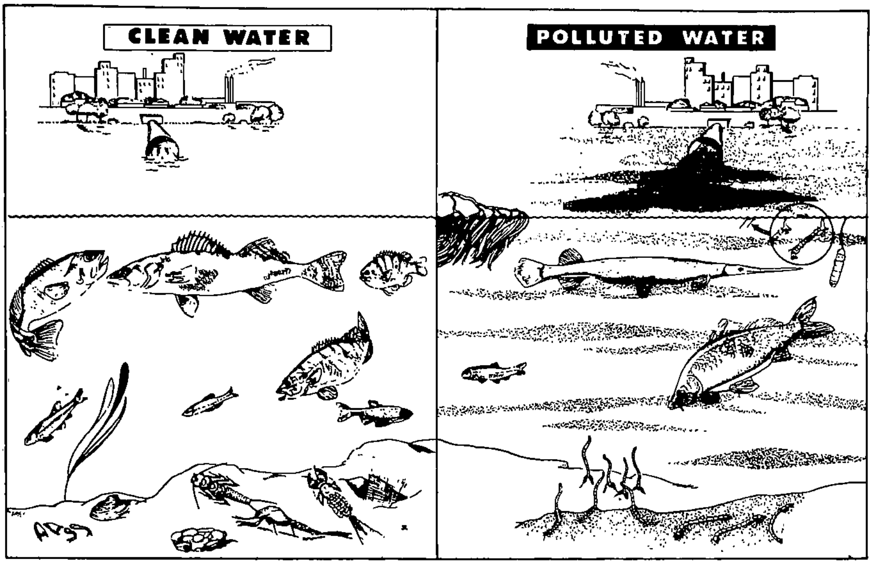



**Figure 1. Pictórial diagram showing water quality effects on aquatic • life [after Ingram, Bartsch, and Jex (10)].**

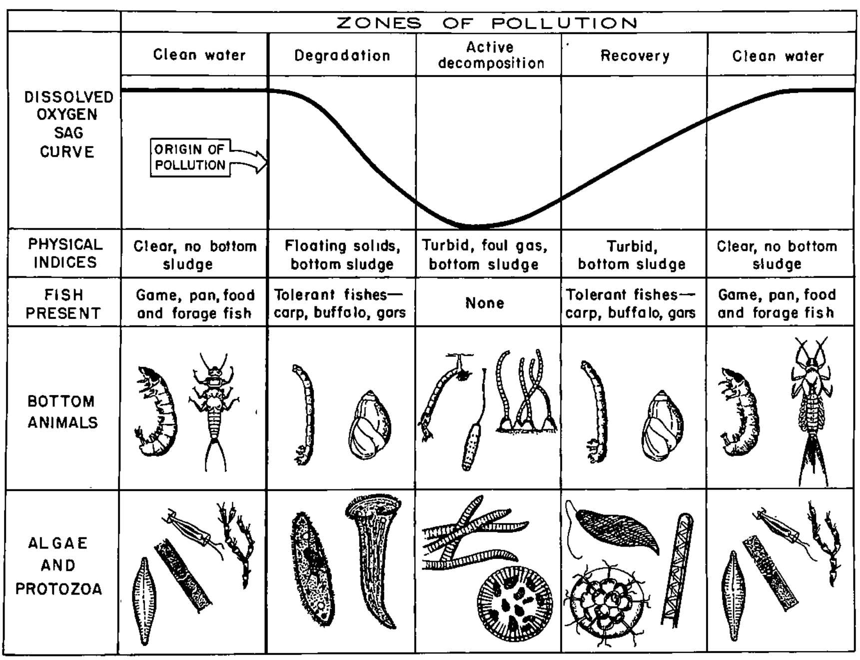



**Figure 2. Pictorial diagram showing some examples of life associated with clean water and water polluted by organic wastes.**

plied is simply this—in the interest of clarity and usefulness, please do not say "Monolassiocoliminophylocumhypophylum carpodendrum [sic: Monolassiocaliminophylorumhypophylum carpodendrum]" when well-known "Joe" or "Bill" will do just as well.

Described in the following paragraphs are methods that have been used effectively to present biological data in water pollution survey reports. One or more of the following graphical expressions, together with pertinent interpretive schemes, make it possible to say "Joe" and "Bill" in a useful way to show important biological relationships. Graphical presentations that can be used effectively to summarize the impact of pollution on stream life include bar graphs, sector diagrams, simple line graphs, photographs, and pictorial diagrams. Some examples of publications especially pertinent to this discussion because they used graphics for effective expression of biological data and are readily available are those by Bartsch (2), Patrick (3), Henderson (4), Surber (5), Ingram (6), Beck (7), Wurtz (8), and Bartsch and Ingram (9).

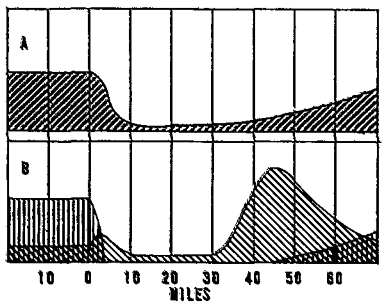
## PICTORIAL DIAGRAMS

Pictorial diagrams such as Figures 1 and 2* have a dual function in communicating biological knowledge effectively. In the clean-up campaign type of water pollution control leaflet or brochure intended for wide distribution to the public, they can be used to show the very general as well as some of the more specific impacts of pollution on living aquatic resources. They also function, and perhaps best, as expressive educational tools for public hearings and meetings, at civic club addresses, and in conservation and nature study courses in the colleges and other levels of education. Figure 1 was developed as a display poster to show, in dramatic fashion, the gross impact of pollution on aquatic life (10). Physical and chemical conditions are shown together with existing aquatic life influenced by them so that interrelations between living conditions and existing life are clearly evident. The poster suggests the kinds of contribution to be made by various sciences when they unite in a joint attack on the problem. This figure has been republished in a number of industrial and conservation journals to emphasize the potential effects of pollution

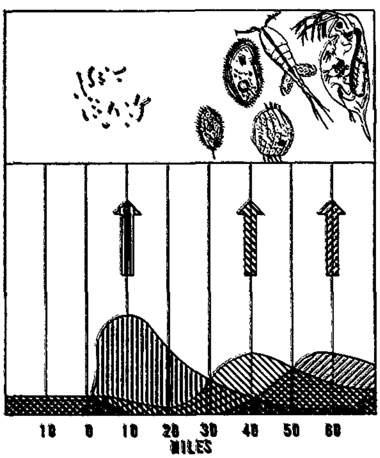
*Figure 2 was modified from a figure in "Environment and Health." PHS Publication No. 84, Federal Security Agency, Public Health Service, Washington, D. C. (1951).

on aquatic life. It was used by Hiram Walker and Sons, Inc. (11) to illustrate pollutional effects in the Illinois River, by conservation agencies of West Virginia (12), Wisconsin (13), and Tennessee (14), and by the Tennessee Department of Public Health (15).

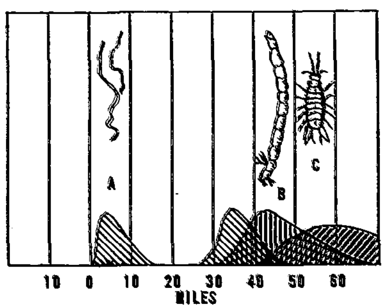
Other pictorial diagrams have been used to illustrate biotic changes resulting from pollution which occur in linear fashion along a stream. In Figure 2, examples of specific characteristic aquatic organisms are shown in relation to stream condition expressed as familiar physical and chemical parameters indicative of heavy pollution by raw sewage. Notable are the dissolved oxygen sag curve and linear distribution of sewage sludge and solids




A. RESPONSES OF BOTTOM ORGANISMS TO ENTRY OF RAW SEWAGE AT ZERO MILEAGE LEVEL. (A) VARIETY DISTRIBUTION: (B) POPULATION ALTERATIONS OF "CLEAN WATER" AND "POLLUTIONAL" BOTTOM FORMS




B. LINEAR ALTERATIONS IN POPULATIONS OF BACTERIA, CILIATE PROTOZOANS, AND CRUSTACEANS




C. LINEAR ALTERATIONS IN POLULATIONS OF SLUDGE-WORMS (A) BLOODWORMS (B) AND SOWBUGS (C)

**Figure 3. Pictorial graphs demonstrating effects of raw sewage on population and kinds of selected organisms [after Bartsch (2)].**

which help to delineate the stream zones shown. This figure is essentially a qualitative one, showing for the fish a shift from desirable kinds to less desirable ones or none. For the bottom animals it shows a replacement of high oxygen demanding insect larvae, incapable of inhabiting sludge, by much less sensitive organisms, such as red "blood worms," sludge worms, rat-tail maggots, and sewage mosquito wrigglers. The algae and protozoa shown are the kinds that one would expect to find dominant in company with the indicated fish and invertebrates.

A quantitative dimension is added to the qualitative distribution of selected organisms in Figure 3. The point of entry of the pollution, which is raw domestic sewage, is designated as zero mile. Figure 3A shows clearly that the entry of pollution causes the variety of bottom organisms to diminish. At some reach downstream (between miles 40 and 50) there is a stimulation of tremendous numbers of one or two species. Figures 3B and 3C show the linear order of dominance of selected organisms.

## Photographs of Organism Samples

Where one is interested in the influence of wastes on the bottom productivity of fish-food organisms, photographs of the collected samples are a simple but highly effective mode of data expression. Without verbal embellishment the essentials of the situation in the stream are evident at a glance. This method was used to advantage by Henderson (4) to show conditions in the South Fork of the Shenandoah River, Virginia, downstream from industries discharging chemical wastes. Results of a later clean-up campaign on the same river can be well shown in this way, also (Figure 4).

## Bar Graphs

Simple bar graphs can be used advantageously in summarizing biotic data to accompany graphic expressions of chemical and physical data. Unlike line graphs, they do not tend to create a false impression that biotic abundance follows a smooth ascending or descending pattern between stations simply because the points are connected by straight lines. Spacing of bars can be so arranged that pertinent watercourse data, such as location of pollution sources or tributaries, can be clearly shown (Figure 5). This figure summarizes simply and concisely seven pages of tabular data dealing with generic names of the organisms found at each of nine stations along 61.9 miles of stream. Station 9 is a control station in a river reach above major sources of municipal and industrial pollution. Station 7*, another control reach is on an unpolluted tributary that joins the Pigeon River between sampling points where maximal depressing effects of pollution on the variety of aquatic life are evident.

Horizontal bar graphs have been used less commonly than the vertical type in stream pollution work. In Figure 6 data, which otherwise would form a series of lengthy tables of the type often hidden in a report appendix, are presented in an interpretive and revealing way. Actually, this chart demonstrates how three parts of a biotic picture can be presented together in a

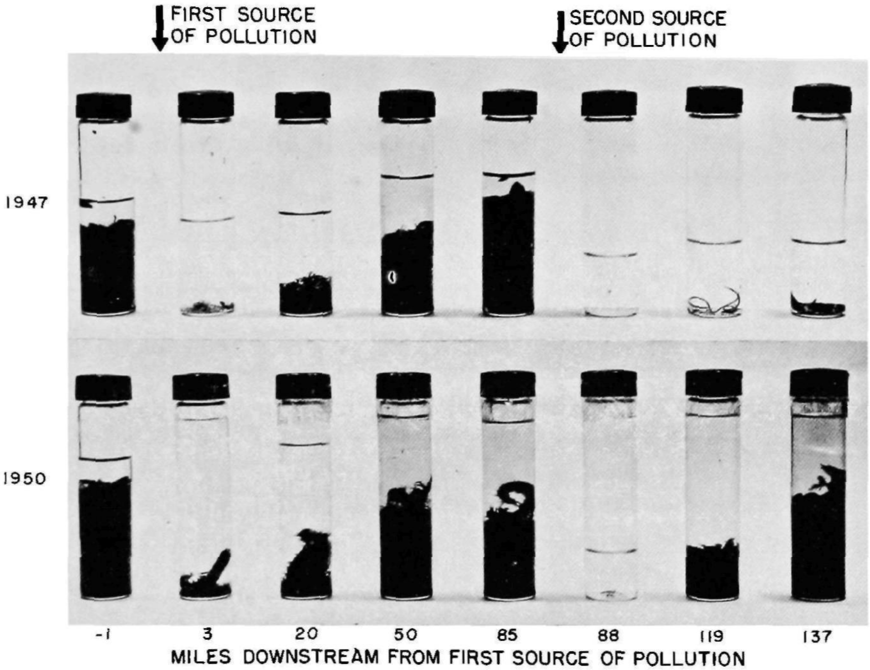



**Figure 4. Photographs of collected samples, showing quantities of fish food organisms per unit area, in South Fork of Shenandoah River, Va. Comparison of two years shows improvements following abatement efforts [after Henderson (4)].**

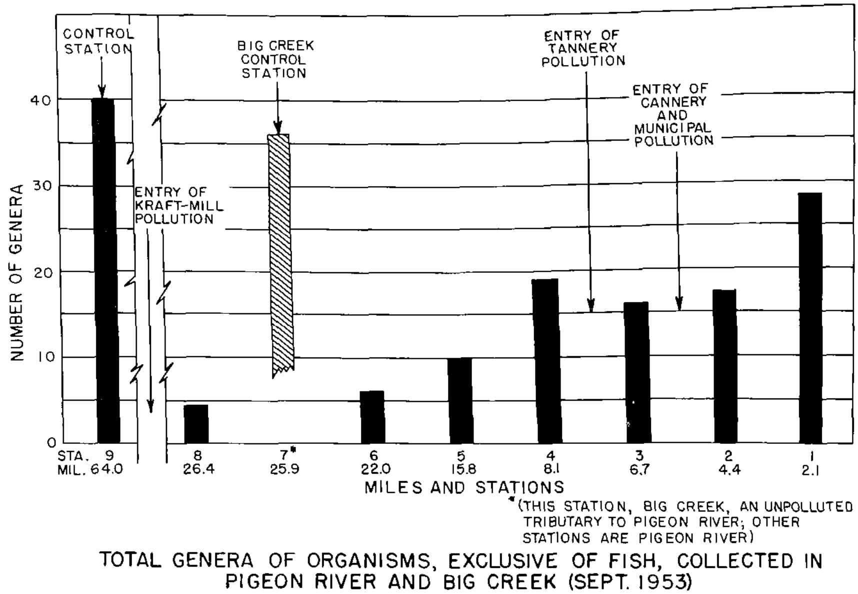



**Figure 5. Vertical bar graph showing total genera of organisms exclusive of fish, collected in Pigeon River and Big Creek, Sept. 1953 [after Mullican, unpublished].**

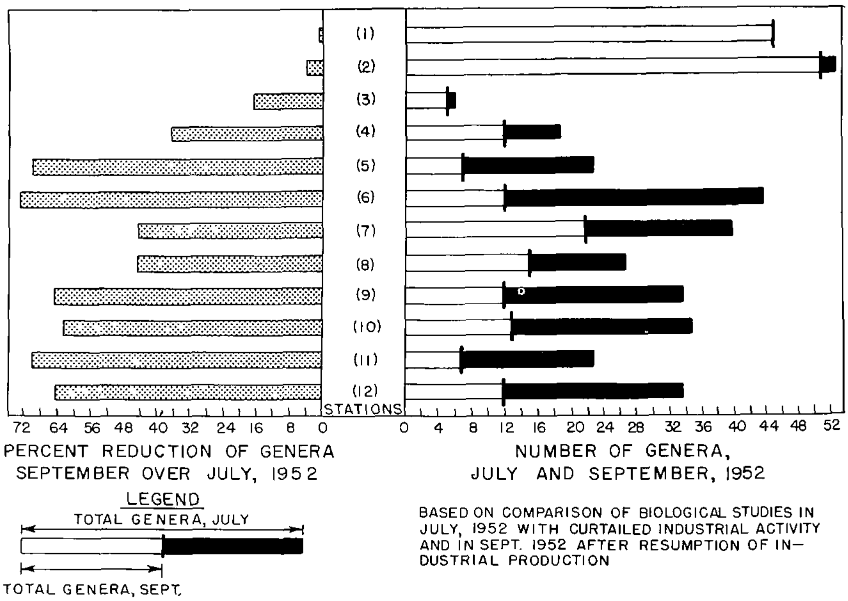



**Figure 6. Horizontal bar graph showing effect of industrial wastes on total genera of organisms in Mahoning River [after Ingram (6)].**

meaningful form where, if done separately, interrelation between organisms, pollution, and waste discharge history would be much less apparent.

Collections of aquatic life on which Figure 6 is based were made in July and September 1952. The July collections were made after the great bulk of industrial production was curtailed by a steel strike along the Mahoning River, Ohio, and while the load of remaining pollution came from untreated municipal sewage. September collections were made at the same stations after the strike was settled and industrial production was resumed. Then the pollution load consisted of both industrial and municipal wastes. Differences in the number of genera of plants and animals under conditions existing in July and September are shown in the right-hand part of the chart. Stations 1 and 2 were control reaches; all other stations were subjected to varying loads of pollution.

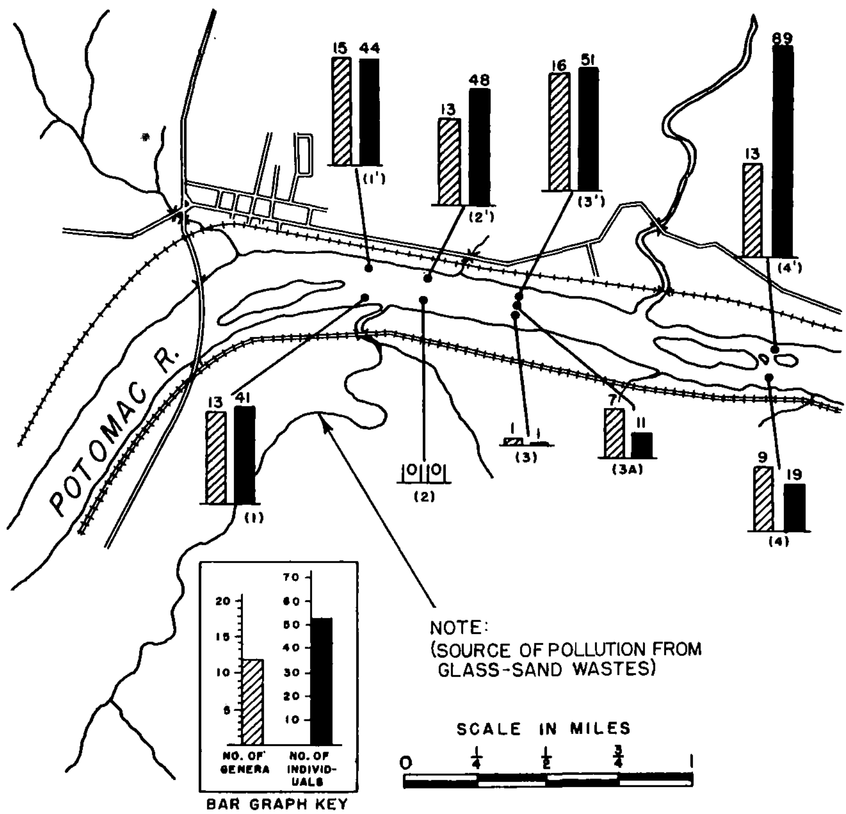



**Figure 7. Vertical bar graphs, superimposed over a map, used to show total genera and individuals of bottom animals per unit area [after Bartsch (16)].**

The left-hand side of the chart shows the per cent reduction of genera in September over July 1952. It is obvious at a glance that the biotic variety of the river was reduced concurrently with resumption of industrial activity and the resulting increased pollution loads reaching the stream. That the indicated reduction is not attributable to seasonal variation of aquatic life is attested by the similarities in generic numbers collected at upstream Control Stations 1 and 2 in both July and September.

Figure 7 demonstrates the use of vertical bar graphs, superimposed over a map of an area, to pinpoint spatially the variation in bottom organisms per square foot of area sampled. This graphic example makes it possible to visualize the influence of settleable solids from a glass sand operation in limiting both the number of kinds and the quantity of individuals of bottom organisms. The wastes are carried down a small creek and enter the river on the south bank between Stations 1 and 2. Stations 1 and 1′ represent upstream control stations. The south half of the stream is the area principally affected by the waste. Bottom organisms are

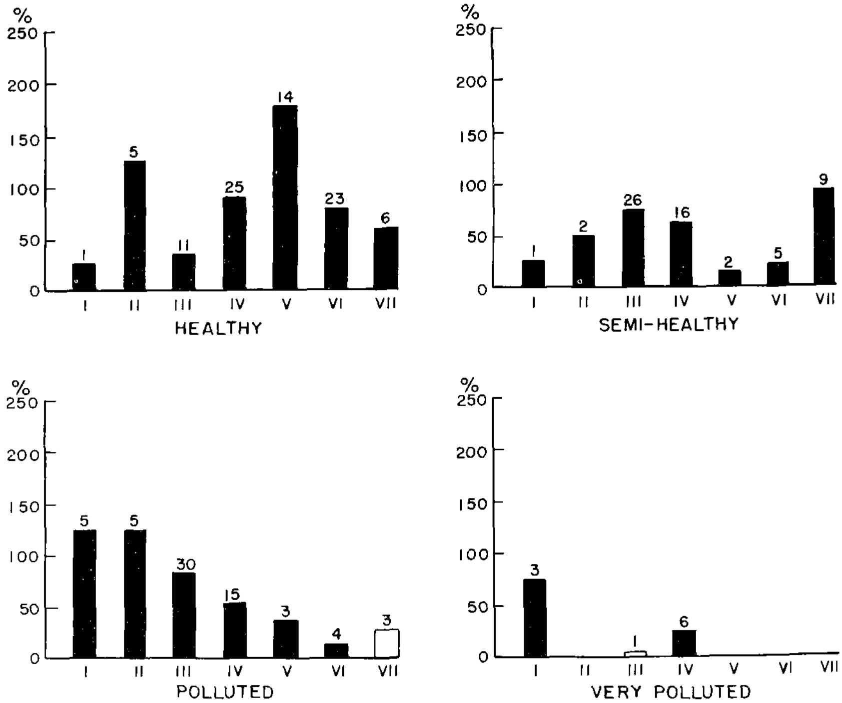



**Figure 8. Histograms, based on selected organisms, illustrating healthy, semi-healthy, polluted, and very polluted stations in Conestoga Basin, Pa. [after Patrick (3)].**

absent at Station 2, and only one was collected at Station 3. A slight increase in kinds and numbers of individuals was noted at Station 4. The stations in the north half of the stream show contrasting abundance of bottom organisms at Stations 1′ to 4′ which are outside the influence of the glass sand waste.

Patrick (3) has used vertical bar graphs to show the presence and variation in abundance of selected species of organisms as related to varying degrees of pollution. Bacteria, fungi, and aquatic flowering plants are omitted from the graphs. When the biotic data are so recorded, ". . . these histograms seem to fall into four general groups which we have designated as healthy, semi-healthy, polluted, and very polluted" (Figure 8). Species of organisms that are associated in any one of the seven columns of a histogram have been grouped together "Because certain groups behaved similarly in response to a given environment . . ." (3). These "groups" that form the columns labeled I to VII are characterized in Table I.

**TABLE I—Classification of Groups of Organisms Shown in Figure 8**

| GROUP | ORGANISM |
|---|---|
| I | Blue-green algae; green algae of the genera *Stigeoclonium, Spirogyra,* and *Tribonema;* the bdelloid rotifers plus *Cephalodella megalocephala* and *Proales decipiens* |
| II | Oligochaetes, leeches, and pulmonate snails |
| III | Protozoa |
| IV | Diatoms, red algae, and "most of the green algae" |
| V | All rotifers not included in Group I, clams, gill-breathing snails, and tricladid flatworms |
| VI | All insects and crustacea |
| VII | All fish |

On the ordinate, the 100-per cent value represents the average number of species for each group found at stations characterized as ". . . typically healthy stations" on the basis of chemical, bacteriological, and biological data. The specific bases for interpreting the histograms to indicate whether they portray healthy, semi-healthy, polluted, or very polluted conditions (Figure 8) are:

1. Healthy Station: Groups IV, VI, and VII are all above the 50-per cent level. Groups I and II ". . . varying greatly depending on the ecological conditions and degree of enrichment of a stream."

2. Semi-healthy Station: (a) Either or both Group VI or

VII are below 50 per cent and Group I or II is under 100 per cent, or (b) Either Group VI or VII is below 50 per cent, and Groups I, II, and IV, are 100 percent or over; or Groups I and II are 100 per cent or over and Group IV is double width. The double width of columns is explained as follows: " 'Semi-healthy' is the common condition in which the balance of life as described for a healthy station has been somewhat disrupted but not destroyed. Often a given species will be represented by a great number of individuals. This condition is noted in the histograms by a double width column."

3. Polluted Station: (a) Either or both of Groups VI and VII are absent, and Groups I and II are 50 percent or greater. (b) Groups VI and VII are both present and below 50 per cent in which case Groups I and II must be 100 per cent or more.

4. Very Polluted Station: (a) Group IV is below 50 per cent and Groups VI and VII are absent, or (b) Groups VI or VII are present and I or II are less than 50 per cent.

Wurtz (8) presents histograms (Figure 9), which deal with selected organisms that are "tolerant" and "non-tolerant" of pollution. "Tolerant" and "non-tolerant" organisms are not listed by scientific names but are shown by their relative abundance. Ranges of pollution intensity reflected in the graphs are not defined.

Each histogram is constructed of four columns, each representing one of the following categories: (a) B: burrowing organisms, (b) S: sessile organisms, (c) F: foraging organisms, and (d) P: pelagic organisms. Protozoa, non-tricladid turbellara, aschelminthes, and entomostracan crustacea are omitted from the histograms. Columns may extend up and down from a baseline. The "non-tolerant" portion of the population is plotted above this line and the "tolerant" portion below it. Wurtz (8) states, "The columns are plotted as a frequency index in which the total number of species found at any station represents a frequency of 100 per cent for that station. Thus the contained area in all the columns of any one histogram equals 100 per cent." He further states that, "In general, the stream may be considered as a clean-water stream when the non-tolerant species represent more than 50 per cent of the population. If the non-tolerant species drop much below this level there is cause for concern as regards stream conditions. The factor that causes this depression can be interpreted as pollution."

For the "normal clean-water station" the four columns making up a histogram, from left to right, would be "approximately" 5, 40, 45, and 10 per cent. These percentages that are stated to exist for a "normal clean-water station," are qualified by station: "However, this is considerably modified by environmental conditions as well as by the effects of pollution. Nevertheless, the proper interpretation of the relative species diversity in each column is the most sensitive method of evaluating stream conditions. Unless gross differences exist between the total number of species at any one station compared with any other station, this feature cannot be used for the interpretation of pollution. It may, however, be considered as supporting evidence for the conclusions drawn as regards any particular station." Wurtz (8) states that clean stations had from 53 to 115 species and polluted stations varied in species from 3 to 46; species in the zones of recovery varied from 25 to 75.

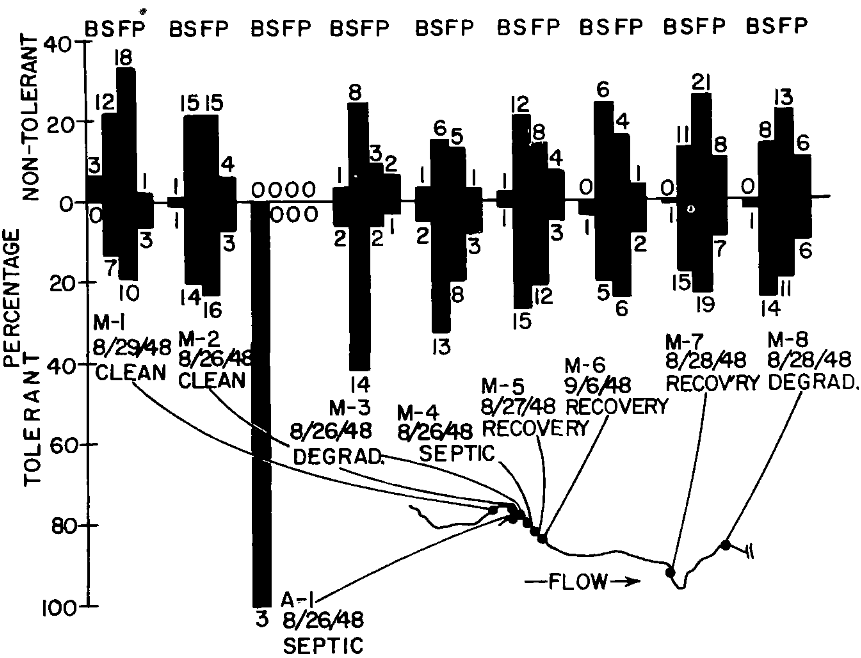



**Figure 9. Histograms, based on selected organisms, illustrating stream reaches of clean, degradation, septic, and recovery conditions [after Wurtz (8)].**

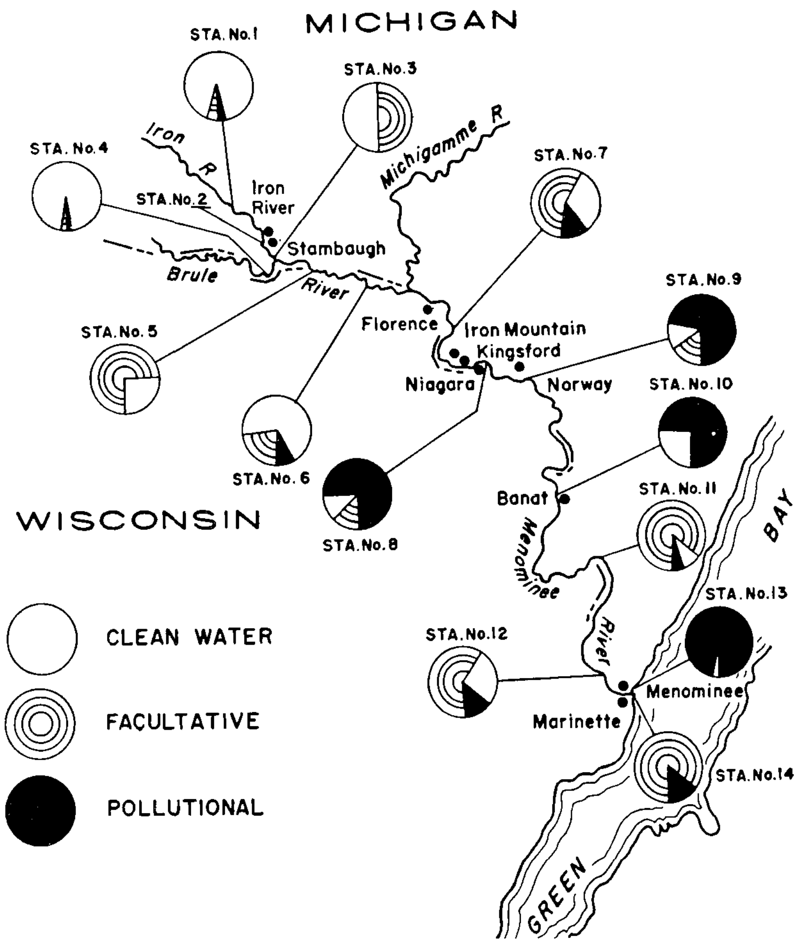



**Figure 10. Sector diagrams showing percentages of clean water, facultative, and pollutional bottom organisms [after Surber (5)].**

## SECTOR DIAGRAMS

Sector diagrams have long been used to express biological conditions in polluted water. A paper by Surber (5) is a recent example in which such diagrams are used to show the abundance of bottom animals that he groups as clean-water, facultative, and pollutional, in studies of the Menominee and Kalamazoo Rivers, Michigan (Figure 10). He states that probably, ". . . only the use of 'facultative' requires explanation: facultative animals are those forms that are able to live in fairly heavily-polluted areas as well as in clean-water situations." This interpretation has not always been used; some workers, unfortunately, have lumped in this category organisms whose responses to pollution are not known. In addition, it is not always clear whether the categories refer to the total number of species or to total population.

## LINE GRAPHS

The line graph, of course, has been the common base for graphical presentation of most data found in reports of water pollution surveys. Too often, no attempt is made to project the significance of long lists of animal and plant names into interpretative graphs that are meaningful to those not familiar with scientific biological names. Through this simple device, however, one can avoid such long lists of names which for apparent lack of meaning are usually entombed in an appendix of the report. Simple line graphs when used where appropriate in place of or to supplement name lists, can prove to be most useful to all who find need to read water pollution survey reports. Such enhancement of bacteriological, chemical, and physical data often could prove to be the yeast that leavens the bread.

In a recent paper, Beck (7) uses simple line graphs to demonstrate the usefulness of a new method for reporting biotic data. This method is one of the few attempts to express biotic condition as a single number. The number, called the "Biotic Index," is derived in two steps:

1. For a given stream station, determine the number of species of organisms that tolerate no appreciable organic pollution (Class I), and the number of species that tolerate moderate organic pollution but cannot exist under near-anaerobic conditions (Class II).

2. Biotic Index = 2(n Class I) + (n Class II).

This Biotic Index may be defined as ". . . an index value based on biological findings and indicative of the cleanliness (with regard to organic pollution) of a portion of a stream or lake." The index may vary from 0 to 40; the index that may be accepted without explanation as indicative of a clean stream is 10; a grossly polluted stream will have an index of 0; and a moderately polluted stream, 1 to 6.

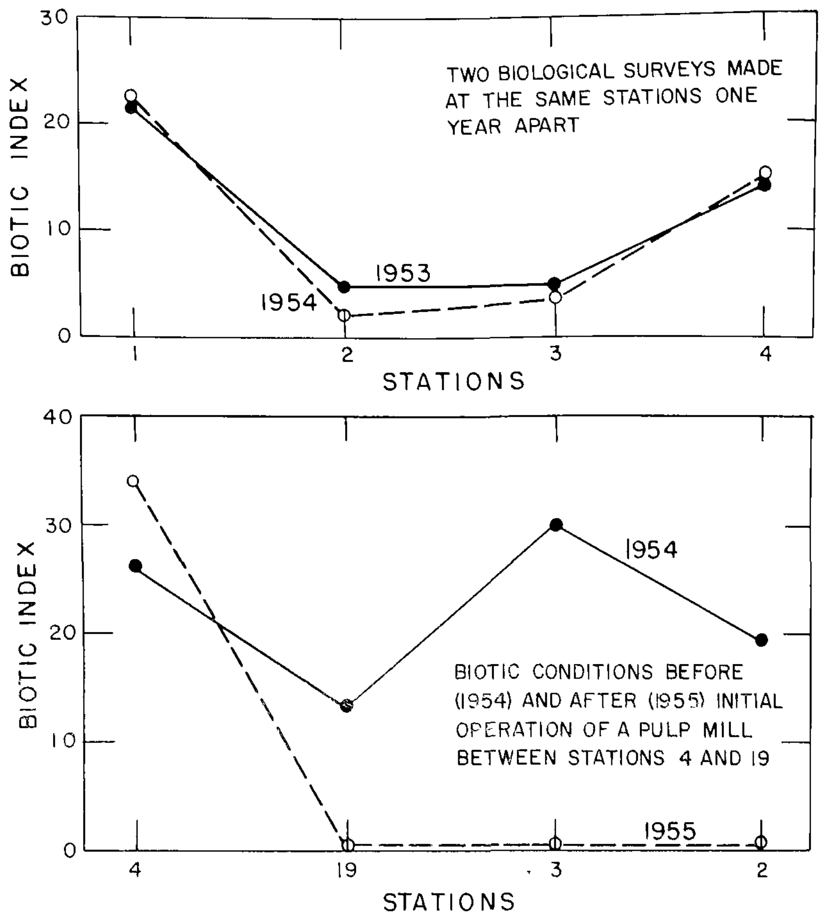



**Figure 11. Presentation of biological data expressed as biotic index [after Beck (7)].**

The clarity with which a graphic plot of biotic indices expresses stream condition is shown in Figure 11.

## ACKNOWLEDGMENT

Appreciation is expressed to the following persons who supplied data for incorporation into this paper: Mr. William M. Beck, Jr., Biologist, Florida State Board of Health; Mr. Harold

Mullican, Chief Biologist, Tennessee Stream Pollution Control; Dr. Ruth Patrick, Curator of Limnology, The Academy of Natural Sciences of Philadelphia; Mr. Eugene W. Surber, Assistant Federal Aid Supervisor, Region IV, U. S. Fish and Wildlife Service, Atlanta, Georgia; Dr. Charles B. Wurtz, Consulting Biologist, Philadelphia, Pennsylvania; and Mr. Croswell Henderson, Water Supply and Water Pollution Research, Robert A. Taft Sanitary Engineering Center of the U. S. Public Health Service.

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*Chapter IV**

# EMPIRICAL EXPRESSION OF ORGANISMS AND THEIR RESPONSE TO ORGANIC POLLUTION IN A FLOWING STREAM

Increased field investigations over the past 10 years, directed toward the abatement of pollution, have prompted this pictorial presentation to show the impact of pollution upon the stream environment and in turn upon the stream life, or biota. The illustrations were developed initially for use in training sanitary engineers and supporting scientists at the U. S. Public Health Service's Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio.

To show schematically the effects of pollution on biota, raw domestic sewage has been chosen as the pollutant. With such a waste, the lowering of dissolved oxygen and formation of sludge deposits are the most commonly seen of the environmental alterations that damage aquatic biota. Fish and the organisms they feed on may be replaced by a dominating horde of animals such as mosquito wrigglers, bloodworms, sludge worms, rattailed maggots and leeches. Black-colored gelatinous algae may cover the sludge and, as both rot, foul odors emerge from the water and paint on nearby houses may be discolored. Such an assemblage of abnormal stream life urges communities not to condone or ignore pollution, but to abate it without delay. This biotic picture emphasizes that pollution is just as effective as drought in reducing the utility of a valuable water resource. They help to make clear that pollution abatement is a vital key to the over-all problem of augmenting and conserving waters of this land.

No two streams are ever exactly alike. In their individualism streams differ from each other in the details of response to

*Published with permission of PUBLIC WORKS MAGAZINE in which the original title was "Stream Life and the Pollution Environment," by Alfred F. Bartsch and William Marcus Ingram, Vol. 90, No. 7, pp. 104-110, 1959: the original illustrations were in color.

the indignity of pollution. In the following paragraphs, and in the charts they describe, the hypothetical stream is made to conform exactly to theory, showing precisely how an idealized stream and its biota should react in a perfect system. In reality, of course, no stream will be exactly like this although the principles shown can be applied with judgment to actual problems that may be encountered.

## ASSUMED CONDITIONS

The stage for discussion is set in Figure 1. The horizontal axis represents the direction and distance of flow of the stream from left to right. Time and distance of flow downstream are shown in days and also in miles. The vertical scale of quantity—or more accurately, concentration—expressed in parts per million, applies to dissolved oxygen and biochemical oxygen demand at distances upstream and downstream from the origin of the sewage discharge, which is identified as point zero. Here, raw domestic sewage from a sewered community of 40,000 people flows to the stream. The volume flow in the stream is 100 cubic feet per second, complete mixing is assumed, and the water temperature is 25°C. Under these conditions the dissolved oxygen (D.O.) sag

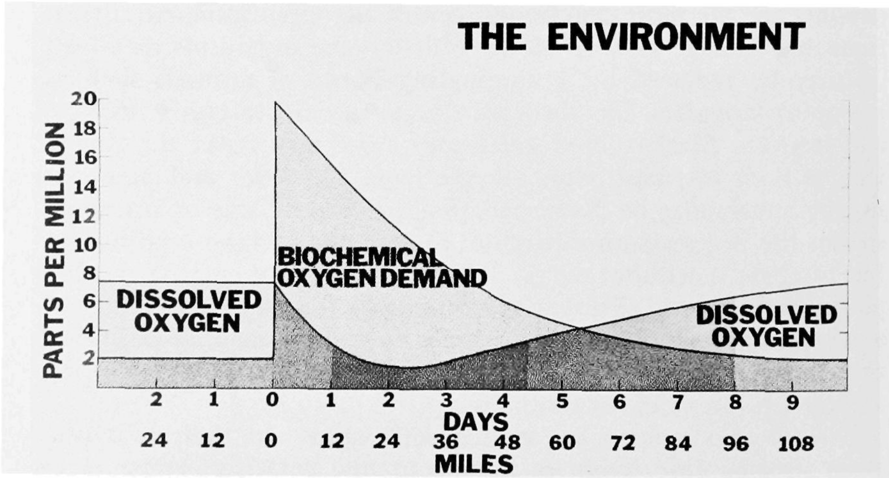


**Figure 1. The assumptions in the hypothetical pollution case under discussion are a stream flow of 100 cfs, a discharge of raw sewage from a community of 40,000 and a water temperature of 25°C, with typical variation of dissolved oxygen and BOD.**

curve reaches a low point after two and one-quarter days of flow and then rises again toward a restoration similar to that of upstream, unpolluted water.

The biochemical oxygen demand (BOD) curve is low in upstream, unpolluted water, increases at point 0 from the great charge of sewage and gradually decreases from this point downstream to a condition suggestive of unpolluted water. BOD and D.O. are so interrelated that the dissolved oxygen concentration is low where BOD is high, and the converse also is true. From left to right the stream zones are: clean water, degradation, active decomposition, recovery, and clean water.

## EFFECTS OF REAERATION

Figure 2 represents an interpretation of the two principal antagonistic factors that have to do with the shape of the D.O. sag curve. The biochemical and other forces that tend to exhaust D.O. supplies, called collectively the process of deoxygenation, would reduce such resources to zero in about a day and one-half if there were no factors in operation that could restore oxygen to water. The river reach where D.O. would be completely gone would occur about 18 miles downstream from the point of discharge of sewage from the municipality. However, with reaeration fac-

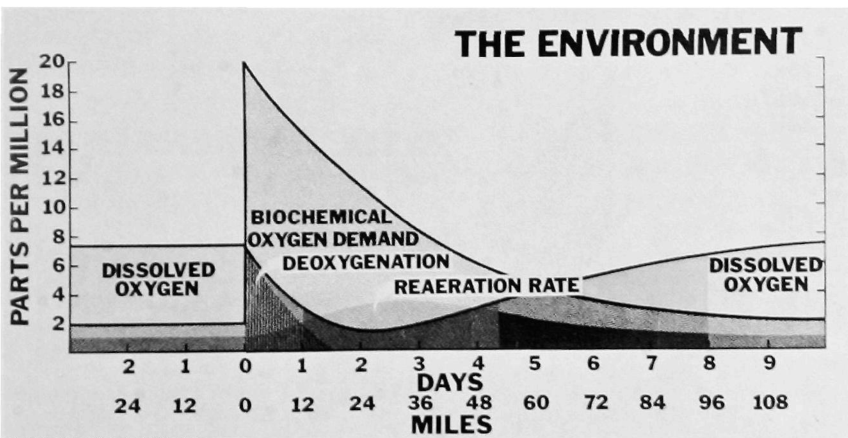


**Figure 2. The dissolved oxygen concentration in the stream is partially destroyed by the pollution load. Full depletion is avoided by reaeration processes.**

tors at work, there is appreciable compensation for deoxygenation, and in this way the actual contour of the oxygen sag curve is determined. Thus, the low point of the curve is not attained at one and one-half days of flow at mile 18 with a zero D.O., but in reality is reached at about two and one-quarter days of flow at about mile 27. The D.O. here does not go to zero, but to 1.5 ppm.

If the population of the city remains fairly uniform throughout the year, and the flow is relatively constant, the low point of the D.O. sag curve can be expected to move up or down the stream with fluctuations in temperature. In winter, one can expect to find the low point farther downstream than shown. In other seasons, if temperatures exceed the 25°C upon which the charts are based, D.O. will be depleted more rapidly and drastically with the low point farther upstream.

The reach of any stream where the D.O. sag curve attains its low point obviously is the stream environment poorest in D.O. resources. It represents a place where aquatic life that may need a high D.O. can suffocate or from which such life may move to other stream areas where the D.O. resources are greater.

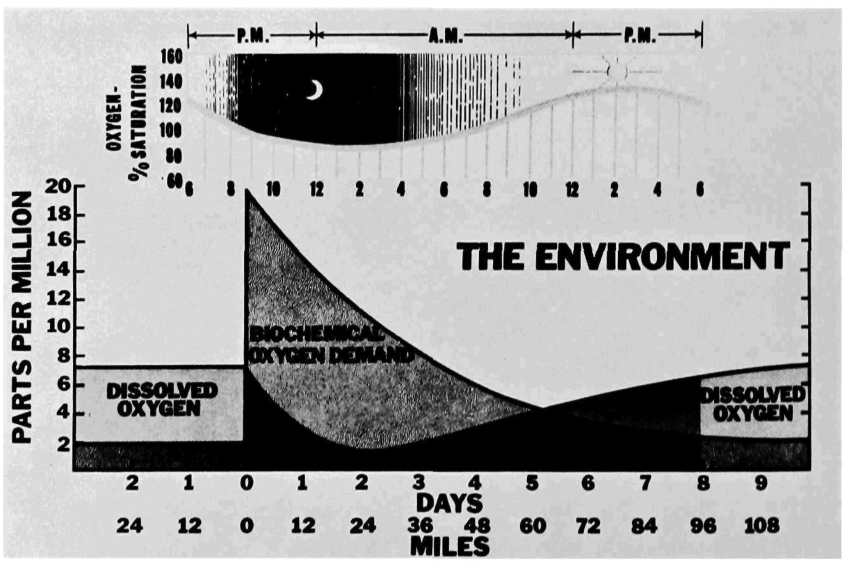


**Figure 3. Dissolved oxygen fluctuates according to available light, a result of photosynthesis. Thus, values on the lower curve are subject to daily variation.**

## EFFECT OF LIGHT

The upper graph of Figure 3 illustrates fluctuations of dissolved oxygen that may occur over a 24-hour period at a single point in a stream with average density of aquatic greenery such as planktonic algae or larger submerged plants. For sake of explanation, any point in the recovery zone would exhibit such diurnal D.O. variations. The lower graph shows only linear changes in D.O., and gives no indication of the daily variation in availability of this vital gas that may occur at any single selected point.

If this selected point is in the recovery zone at mile 72, one can see from Figure 3 that D.O. varies from a low of about 80 percent saturation at 2:00 a.m. to about 140 percent at 2:00 p.m. Diurnal variation such as this is a result of photosynthesis chiefly in algae but in other plants also. During daylight hours these plants give off oxygen into the water in such large quantities that if the organic wastes are not sufficient to use up much of the D.O. in oxidizing sewage, the water commonly becomes supersaturated at some time during daylight hours. In addition to giving off oxygen, the photosynthetic process results in the manufacture of sugar to serve as the base from which flows the nutritional support for all stream life. The process of photosynthesis can be illustrated schematically as:

$$6\,CO_2 + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

This action proceeds through the interaction of the green pigment, chlorophyll, contained in living plant matter, of sunlight, carbon dioxide, and even water to form the raw materials into a simple sugar and surplus oxygen.

While photosynthesis occurs, so also does respiration which proceeds 24 hours on end irrespective of illumination. In this well-known process $O_2$ is taken in and $CO_2$ is given off. The algae, during daylight may yield an excess of oxygen over and above their respiratory needs, the needs of other aquatic life, and the needs for the satisfaction of any biochemical oxygen demand. Under these conditions, surplus oxygen may be lost to the atmosphere. During hours of darkness photosynthesis does not occur and gradually, the surplus D.O. that was present is used up or reduced by algae, fish, various insects, clams, snails and other aquatic life in respiration, and by bacteria in satisfaction of the BOD. That is why oxygen resources are poorest during early morning hours. During hours of darkness, a stream is typically dependent on physical reaeration for its oxygen resources after exhaustion of the "bank of dissolved oxygen," that was elevated to supersaturation levels by aquatic plants.

Obviously, on stream sanitary surveys where organic wastes such as domestic sewage are pollutants, it is important to sample each station over 24 hours at intervals that are appropriate to reveal information on diurnal D.O. variations. If this is not done and station 1 is sampled consistently around 8:00 a.m. and station 6 around 5:00 p.m. over a weekly or a monthly survey, critical D.O. concentrations will not be found. If interval sampling over 24 hours cannot be done because of workday restrictions, reversing the time of sampling from the upstream to the downstream station on alternate days will at least show variations of D.O. that one can expect through an 8-hour workday.

## Effect of Organic Matter

The bottom graph of Figure 4 illustrates reasons for the decrease in the BOD curve progressively downstream and offers an explanation for the depression in the oxygen sag curve. On this

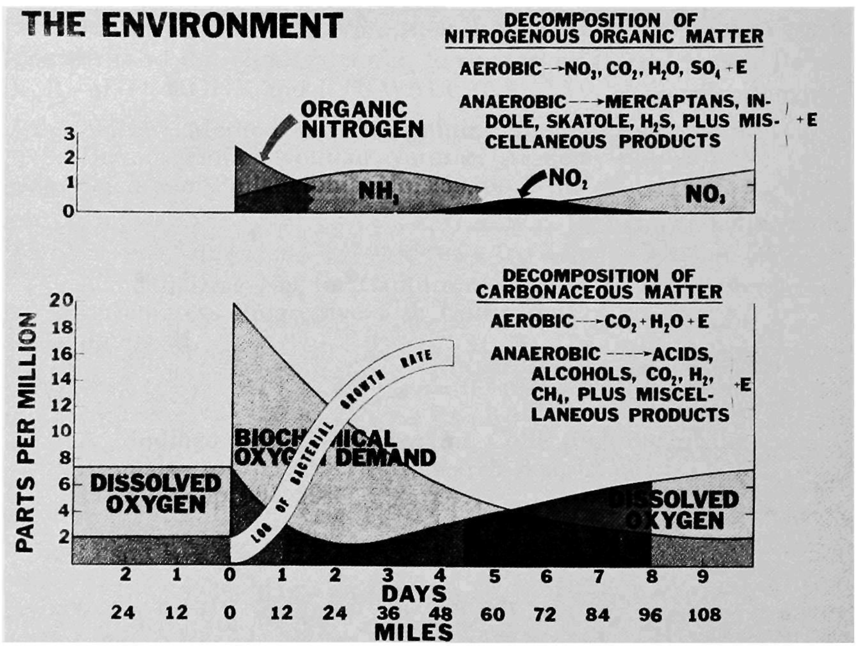


**Figure 4. With a heavy influx of nitrogen and carbon compounds from sewage, the bacterial growth rate is accelerated and dissolved oxygen is utilized for oxidation of these compounds. As this proceeds, food is "used up" and the BOD declines.**

graph there has been superimposed, in white, the shape of the log curve of bacterial growth rate. Accelerated bacterial growth rate is a response to rich food supplies in the domestic raw sewage. During rapid utilization of food, bacterial reproduction is at an optimum, and utilization of D.O. becomes fairly proportional to the rate of food oxidation.

The upper graph illustrates, in principle, the progressive downstream changes in nitrogen from the organic form to the nitrate form. It demonstrates the initial high consumption of oxygen by bacteria that are feeding on proteinaceous compounds available in upstream waters in freshly discharged domestic sewage. With fewer and fewer of these compounds left in downstream waters, the BOD becomes reduced and the D.O. increases. Fat and carbohydrate foodstuffs rather than proteins could have been chosen just as well to show this phenomenon.

The nitrogen and phosphorus in sewage proteins can cause special problems in some receiving waters. Experience has shown that increasing the amount of these elements in water can create conditions especially favorable for growing green plants. In free flowing, clear, pebble brooks they appear as green velvety coatings on the stones or as lengthy streamers waving gently in the current. They are not unattractive and even, in the poetry of Nature, are complimented by the name "mermaid's tresses." These plants are not like the troublesome ones which occur mostly in more sluggish streams, impoundments or lakes, especially when they are artificially fertilized by sewage. In the clean brook, they not only are attractive and natural to see, but also they are a miniature jungle in which animals of many kinds prey upon each other with the survivors growing to become eventual fish food.

In more quiet waters, the algal nutrients in sewage are picked up for growth by less desirable kinds of algae. With great supplies of nitrogen and phosphorus made available, free-floating, minute blue-green algae increase explosively to make the water pea soup green, smelly and unattractive. In some unfortunate localities, nuisance blooms of algae have become so objectionable that waterfront dwellers have had to forsake their homes and see their property depreciate in value. The problem has been studied at a number of localities, and some studies are still in progress. Special legislation has even been formulated requiring that sewage treatment plant effluents not be discharged to susceptible lakes solely because of the algal nutrients they contain. Sometimes, under conditions not well understood, some blue-green algae develop poisons capable of killing livestock, wildlife and fish. Fortunately, such occurrences are rare. It is completely clear that sewage disposal and biological responses of even such lowly plants as algae go hand-in-hand sometimes to plague the desires of man.

## AQUATIC PLANTS

In the lower part of Figure 5 a profile is shown of the water and stream bed with the vertical scale of the latter exaggerated. Sludge deposits begin to accumulate just below the point of sewage discharge. These deposits reach their maximum thickness near the point of origin but blanket the stream bed for many miles downstream. The substance of the deposits gradually is reduced by decomposition through the action of bacteria, moulds and other sludge-dwelling organisms, until it becomes insignificant about thirty miles below the municipality.

Also, at the outfall the water is turbid from fine solids held in suspension in the flowing water. Larger floating solids, destined to sink eventually to the stream bed as settleable solids, are visible

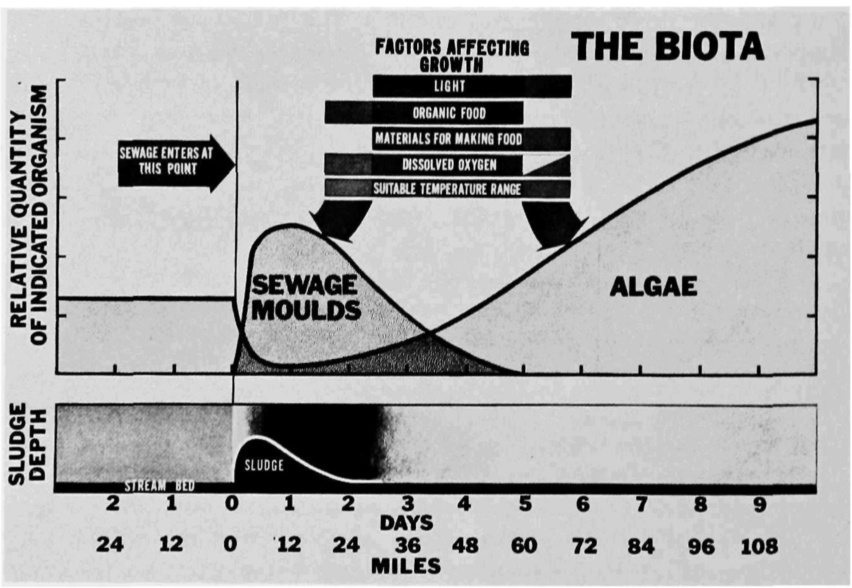


**Figure 5. Shortly after sewage discharge, the moulds attain maximum growth. These are associated with sludge deposition shown in the lower curve. The sludge is decomposed gradually; as conditions clear up, algae gain a foothold and multiply.**

on the water surface as they drift downstream. Both the fine and large solids contribute to the sludge deposit, and as they settle progressively to the bottom of the stream bed, the water becomes clear and approaches the color and transparency of upstream water above the point of sewage discharge.

The upper graph illustrates the relative distribution and quantities of algae, various moulds, and filamentous bacteria such as *Sphaerotilus*. From mile 0 to mile 36, high turbidity from floating debris and suspended solids is not conducive to algal production. Thus, except for slimy blue-green marginal and bottom types, algae are sparse in this reach. In order to grow well algae need sunlight, and here it cannot penetrate the water effectively. Also, floating solids that settle out of the water carry to the bottom with them floating algae that drift into the polluted zone from clear water areas upstream.

Blue-green algae that may cover marginal rocks in slippery layers and give off foul odors upon seasonal decay masquerade under the names: *Phormidium*, *Lyngbya*, and *Oscillatoria*. Green algae that accommodate themselves to the putrid zone of active decomposition frequently include *Spirogyra* and *Stigeoclonium*. *Gomphonema* and *Nitzschia* are among the diatoms that are present here.

Algae begin to increase in numbers at about mile 36. Plankton, or free-floating forms, steadily become more abundant and reach their greatest numbers in algal blooms some 40 to 60 miles farther downstream. This is where reduced turbidity, a lack of settleable sewage solids, final mineralization of proteinaceous organics to nitrate-nitrogen fertilizers, and favorable oxygen relations result in an ideal environment for growth of abundant aquatic plants.

Algae that may be found abundantly here may be represented by the bluegren genera *Microcystis* and *Anabaena;* the pigmented flagellates are represented by *Euglena* and *Pandorina;* the green algae by *Cladophora*, *Ankistrodesmus*, and *Rhizoclonium;* and diatoms by *Meridion* and *Cyclotella*. Rooted, flowering, aquatic plants that form underwater jungles here are represented by the "water pest," *Elodea*, and various species of pond weeds known as *Potomogeton*. Such aquatic forests and meadows present an excellent natural food supply for the aquatic animals, and also serve them with shelter. Thus, commonly as plants respond downstream in developing a diversified population in the recovery and clean water zones, animals follow a parallel development with a great variety of species. In such reaches where the stream consists of numerous alternating riffles and pools, a great variety of fish are likely to occur.

In the reach where algae are scare [sic: scarce], from about mile 0 to mile 36, various moulds and bacteria are the dominant aquatic plants. *Sphaerotilus* filaments may abound in riffle areas at about mile 36 where physical attachment surfaces are available and where oxygen, although low, is adequate. Bacterial slimes may cover rocks and other submerged objects and bank margins. Such slimes have an abundant supply of available food in readily usable form of carbohydrates, proteins and fats and their digestion products. They are not bothered especially by high turbidities or by settleable solids. They do well living in the center of sludge or near it, in what to them is an "apple-pie" environment.

## Bacteria and the Ciliates

Associated with the bacterial slimes are certain ciliated protozoans that feed on bacteria and engulf small particles of settleable organic matter. Such ciliates are also found in aeration tanks of sewage treatment installations as a component of activated sludge and on the surface of rock in trickling filter beds. Common ones are *Epistilis, Vorticella, Colpidium,* and *Stentor.*

Figure 6 illustrates the interrelations between bacteria and animal plankton, such as ciliated protozoans, rotifers and crustaceans. The quantities shown and the die-off curves for sewage bacteria in toto and for coliform bacteria separately are theoretically accurate. The center curve for ciliated protozoans and the last curve representing rotifers and crustaceans are more accurate in principle than in actual quantities.

After entering the stream as a part of the sewage, bacteria, including coliforms, reproduce to become abundant in an ideal environment. Here they feed on the rich organic matter of sewage and by multiplying rapidly offer a ready food supply for ciliated protozoans which are initially few in number. After about a day of flow the bacteria may be reduced through natural die-off and from the predatory feeding by protozoans. After about two days of flow, the stream environment becomes more ideal for the ciliates, and they form the dominant group of animal plankton. After seven days, the ciliates fall victim to rotifers and crustaceans which represent the principal microscopic animal life in the stream.

It has been long suspected that the efficiency of this sewage-consuming biological machine depends upon a close-knit savage

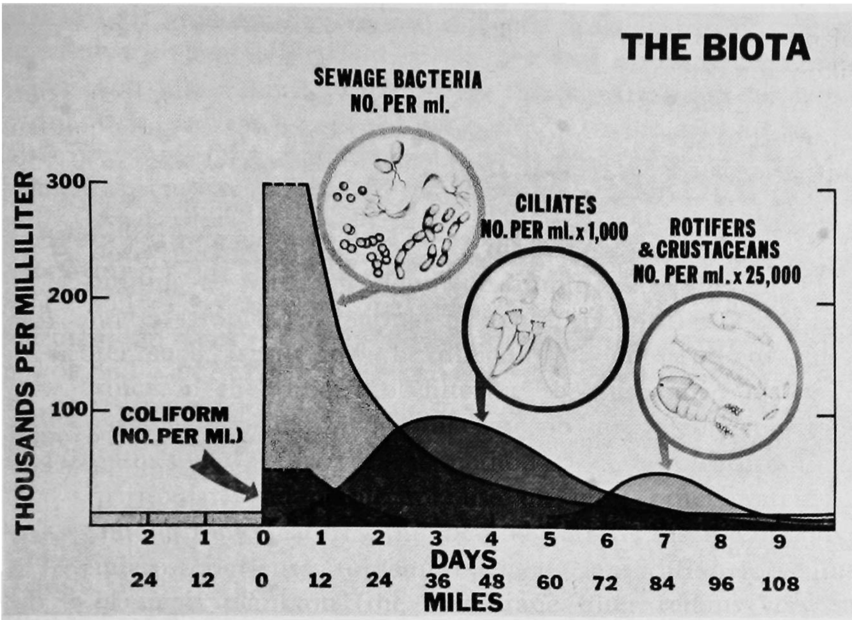


**Figure 6. Bacteria thrive and finally become prey of the ciliates, which in turn are food for the rotifers and crustaceans.**

society in which one kind of organism captures and eats another. Classical research of some time past showed that a single kind of bacterium mixed with sewage in a bottle could not do an efficient or rapid job of breaking down the sewage. Several kinds could do a better job, supposedly because one bacterial type, in acting upon parts of the sewage as food, prepared it for acceptance by another. With several bacteria a multilateral attack was made possible. But even a system like this is inefficient. Bacteria work best only when they are growing rapidly and they do this when they multiply frequently by splitting into two. It is important then that they not be permitted to attain a stable high and lazy population. In the bottle the task of stabilizing sewage goes most rapidly when ferocious bacteria-eating ciliates are introduced to keep the population at a low and rapidly growing state.

These relations between the bacteria eaters and their prey, discovered in the bottle, apply as well to efficient functioning of a modern sewage treatment plant. In some sewage treatment plants, examination is made routinely to see how the battle lines are drawn up between the bacteria eaters and their prey. It now becomes more obvious why sewage disappears so efficiently from the stream. It also is clear why the bacteria, the ciliates, the rotifers and the crustaceans increase, persist for awhile, and then decrease along the course of passage of the stream.

## The Higher Forms

Figure 7 illustrates the types of organisms and the numbers of each type likely to occur along the course of the stream under the assumed physical conditions that were stated earlier. The upper curve represents the numbers of kinds or species of organisms that are found under varying degrees of pollution. The lower curve represents the numbers of individuals of each species. In clean water above the city a great variety of organisms is found with very few of each kind represented. At the point of waste entry the number of different species is greatly reduced, and they are replaced by a different association of aquatic life. This new association demonstrates a severe change in environment that is drastically illustrated by a change in the species make-up of the biota. However, this changed biota, represented by a few species, is accompanied by a tremendous increase in the numbers of individuals of each kind as compared with the density of population upstream.

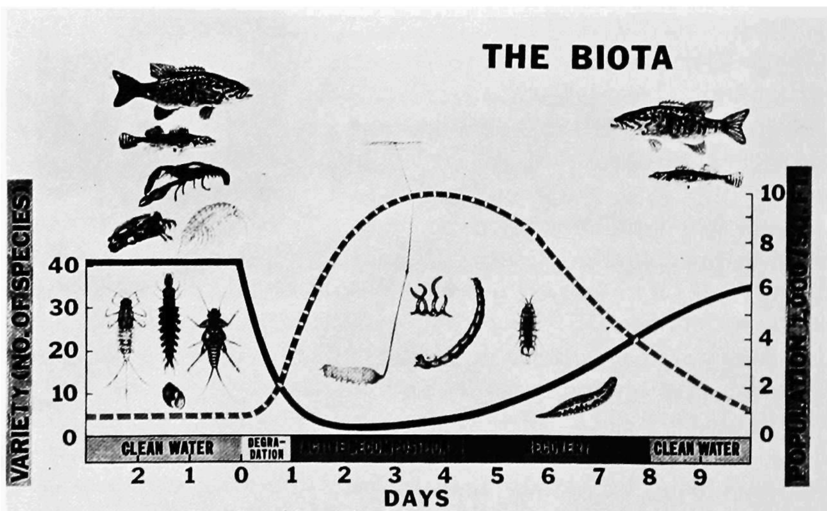


Figure 7. The [upper] curve shows the fluctuations in numbers of species: the [lower] the variations in numbers of each.

In clean water upstream there is an association of sports fish, various minnows, caddis worms, mayflies, stoneflies, hellgrammites, and gill-breathing snails, each kind represented by a few individuals. In badly polluted zones the upstream association disappears completely or is reduced, and is replaced by a dominant animal association of rattailed maggots, sludge worms, bloodworms and a few others, represented by great numbers of individuals. When downstream conditions again resemble those of the upstream clean water zone, the clean water animal association tends to reappear and the pollution tolerant group of animals becomes suppressed. Thus, clean water associations of animals may form parameters around polluted water reaches. Such associations may be indicative that water is fit for multiple uses, while the presence of a pollution tolerant association of animals indicates that water has restricted uses.

Pollution tolerant animals are especially well adapted to life in thick sludge deposits and to conditions of low dissolved oxygen. The rattailed maggot, *Eristalis tenax*, is not dependent on oxygen in water. This animal shoves its "snorkle-like" telescopic air tube through the water surface film to breathe atmospheric oxygen. Thus, even in the absence of oxygen it is one of the few survivors where most animals have suffocated. Those who have worked around sewage treatment installations have probably observed the flesh or milkish colored rattailed maggot in the supernatant over sludge beds where dewatering performance was poor. Commonly associated with it in this supernatant over sludge beds are the immature stages of the well-known "sewagefly," *Psychoda*, and wrigglers of the sewage mosquito, *Culex pipiens*. The rattailed maggot turns into a black and brownish banded fly about three-quarters of an inch long, called a "bee fly" because it closely resembles a bee. It differs by having two wings instead of four and does not sting. Sludge worms, *Tubifex*, are dependent upon the dissolved oxygen in water; however, they are well adjusted to oxygen famine and commonly are found in water with as little as half a part per million. They are actually aquatic earthworms, cousins of the terrestrial earthworms found in lawns and used as fish bait. These worms feed on sludge by taking it into the digestive tract. In passing it through their alimentary canal, they remove organic matter from it, thus reducing the biochemical oxygen demand. Sludge worms one and one-half inches long and as thick as a needle have been observed to pass fecal pellets totaling five feet nine inches through the digestive tract in 24 hours. Fecal pellets that are extruded from the anal openings have on occasion been found to have a biochemical oxygen demand of one-half of that of sludge that was not "worked-over" by them. The sludge worms are then, "actually crawling BOD," in that they incorporate sugars, proteins and fats that are present in sludge into their body cellular components. It may be difficult to visualize the magnitude of BOD removal that one worm, needle-thick in size and one and one-half inches long, can accomplish in relation to an extensive sludge deposit. However, when it is realized that from 7,000 to 14,000 of these worms may be found per square foot of bottom surface in sludges, considerable work is done in removing BOD. By the same token, for example, wrigglers of sewage mosquitoes. [*Sic:* ,] *Culex pipiens,* that feed on the organics of sewage and emerge as adults to fly out of water represent BOD removed. In this instance it is "flying BOD" that is factually taken out of water, whereas the crawling BOD of sludge worms is not removed, but is recycled back as the worms die.

The worm-like body of organisms composing the pollution tolerant association of the rattailed maggot, sludge worms, blood worms, and leeches is an ideal type to have for successful living in sludge. As settleable solids fall to the bottom, such organisms are not trapped and buried in them to die, but by wriggling with their worm-like cylindrical bodies, manage to maintain their position near the surface of sludge in communication with the water interface. Sow-bugs that are shown in Figure 7 with the "wormy-

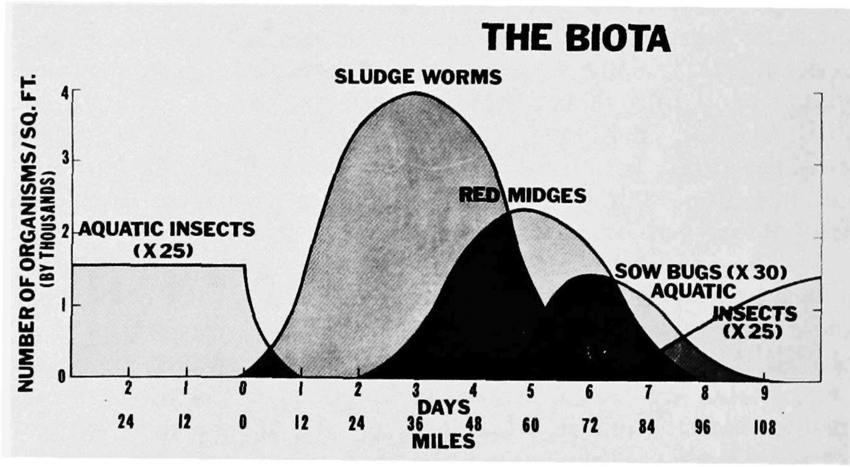


**Figure 8. The population curve of Figure 7 is composed of a series of maxima for individual species, each multiplying and dying off as stream conditions vary.**

horde" do have well-developed appendages, but their life may be marginal on stream bank areas and on the surface of rocks protruding from sludge covered bottoms. Thus, they are not buried by settleable solids.

The invertebrates shown in clean water do not form successful populations in streams where settleable solids sink to form sludge deposits. Because their appendages may become clogged with sludge as solids settle, they may be carried readily to the bottom and be buried alive.

## POPULATION FLUCTUATION

Figure 8 shows that the population curve of Figure 7 is actually composed of a series of population maxima for individual species. The species form a significant pattern in reference to each other and to the varying strength of the pollutant as it decreases progressively downstream. Sludge worms such as *Tubifex* and *Limnodrilus* can better withstand pollution than other bottom invertebrates. Thus, they reach great numbers closer to the source than other bottom dwelling animals. In turn they are replaced in dominance by red midges, also called bloodworms or *Chironomids,* and then by aquatic sow-bugs, *Asellus.* The sludge worms and red midges are so numerous in contrast to the other organisms shown in Figure 8 that numbers of the latter are exaggerated 25 and 30 times to permit showing them effectively. Finally, when the effects of pollution have largely subsided in the environment, a variety of insect species represented by few individuals of each dominates the bottom habitat.

The story of pollution told here emphasizes that stream pollution and recovery may follow an orderly scheme under the influence of interacting physical, chemical and biological forces. Using streams as dumping places for sewage triggers the environmental and biotic changes that have been shown. These changes are not desirable. In most cases, in addition, they are hazardous to public health and otherwise impair the usefulness of valuable water resources. The needed remedy is to confine all of these interacting forces in an acceptable sewage treatment works so that this example of the Nation's water resources is protected for present and future use.

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*Chapter V*

# SELECTED BIOLOGICAL REFERENCES ON RESPONSES OF ORGANISMS TO GROSS POLLUTION

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*Chapter VI*

# DATA ANALYSES AND INTERPRETATION

The biologist is routinely confronted with data analyses and interpretation. Through the years some pieces of information have been very helpful, and these are presented in this chapter. These pieces are grouped broadly into sections pertaining to conversion factors, a chemical dosage chart for the chemical treatment of water, techniques for plankton counting, bio-assay bench charts, and selected references on data collection and analyses. Sub-groups, listed alphabetically, are to be found in the conversion factor section. The conversion factors that are presented have been selected from a large number and hopefully will contain many of those that are used often by the biologist, but not sufficiently routine to be retained in memory.

## CONVERSION FACTORS

### ACRES

| | | |
|---|---|---|
| Hectares | × 2.471 | = Acres |
| Square Meters | × 2.471 × $10^{-4}$ | = Acres |
| Acres | × 4047 | = Square Meters |
| Acres | × 43,560 | = Square Feet |
| Acre-feet | × 325,851 | = Gallons |
| Grams Per Square Meter | × 8.922 | = Pounds Per Acre |
| Kilograms Per Hectare | × 0.8922 | = Pounds Per Acre |
| Milligrams Per Square Centimeter | × 89.22 | = Pounds Per Acre |
| Milligrams Per Cubic Meter | × 2.72 × $10^{-3}$ | = Pounds Per Acre-foot |

## AREA

| | |
|---|---|
| Circle | —Square of the diameter × 0.7854. |
| Rectangle | —Length of the base × height. |
| Sphere | —Square of the radius × 3.1416 × 4. |
| Square | —Square the length of one side. |
| Trapezoid | —Add the two parallel sides × height ÷ 2. |
| Triangle | —Base × height ÷ 2. |

## CIRCUMFERENCE

Circle—Diameter × 3.1416

## DISSOLVED OXYGEN

| | | |
|---|---|---|
| Micromoles Per Liter | $\times 32 \times 10^{-6}$ | = Grams Per Liter |
| Millimoles Per Liter | $\times 32$ | = Milligrams Per Liter |
| Micromoles Per Microliter | $\times 32 \times 10^{3}$ | = Milligrams Per Liter |
| Millimoles Per Square Meter | $\times 2848 \times 10^{-4}$ | = Pounds Per Acre |

## DOSAGE FORMULA (Chemical)

Length (ft.) × Width (ft.) × Average Depth (ft.) × 62.4 × Desired Concentration in ppm $\times 10^{-6}$ = Pounds of Active Material Needed

Speed of Boat (feet per hour) × Width of Spray Pattern (feet) × Depth of Calculated Treatment (feet) × 62.4 × Desired Concentration in ppm $\times 10^{-6}$ = Pounds of Active Material Needed Per Hour

## FEET

| | | |
|---|---|---|
| Fathoms | $\times 6.08$ | = Feet |
| Meters | $\times 3.281$ | = Feet |
| Acres | $\times 43{,}560$ | = Square Feet |
| Square Meters | $\times 10.76$ | = Square Feet |
| Square Feet | $\times 929 \times 10^{-4}$ | = Square Meters |
| Gallons | $\times 1337 \times 10^{-4}$ | = Cubic Feet |
| Cubic Feet | $\times 7.48$ | = Gallons |
| Gallons Per Minute | $\times 2.228 \times 10^{-3}$ | = Cubic Feet Per Second |
| Cubic Feet Per Second | $\times 448.8$ | = Gallons Per Minute |
| Million Gallons Per Day | $\times 1.547$ | = Cubic Feet Per Second |
| Cubic Feet Per Second | $\times 6463 \times 10^{-4}$ | = Million Gallons Per Day |

## GALLONS

| | | | |
|---|---|---|---|
| Acre-feet | × | 325,851 | = Gallons |
| Cubic Feet | × | 7.48 | = Gallons |
| Liters | × | $2642 \times 10^{-4}$ | = Gallons |
| Pounds of Water | × | $1198 \times 10^{-4}$ | = Gallons of Water |
| Gallons Per Minute | × | 1440 | = Gallons Per Day |
| Gallons of Water | × | 8.345 | = Pounds of Water |
| Gallons | × | $1337 \times 10^{-4}$ | = Cubic Feet |
| Gallons | × | 231 | = Cubic Inches |
| Gallons | × | 3.785 | = Liters |
| Cubic Feet Per Second | × | $6463 \times 10^{-4}$ | = Million Gallons Per Day |
| Cubic Feet Per Second | × | 448.8 | = Gallons Per Minute |
| Gallons Per Minute | × | $2.228 \times 10^{-3}$ | = Cubic Feet Per Second |
| Million Gallons Per Day | × | 1.547 | = Cubic Feet Per Second |
| Parts Per Million* | × | 8.345 | = Pounds Per Million Gallons |

## MILES

| | | | |
|---|---|---|---|
| Kilometers | × | $6214 \times 10^{-4}$ | = Miles |
| Statute Miles | × | 1.15 | = Nautical Miles |
| Miles | × | 1.609 | = Kilometers |
| Nautical Miles Per Hour | × | 1.0 | = Knots |

## PARTS PER MILLION

| | | | |
|---|---|---|---|
| Milligrams Per Liter | × | 1.0 | = Parts Per Million |
| Grams Per Liter | × | 1000 | = Parts Per Million |
| Micrograms Per Liter | × | $10^{-3}$ | = Parts Per Million |
| Micrograms Per Gram | × | 1.0 | = Parts Per Million |
| Cubic Centimeters Per Liter | × | 1.4545 | = Parts Per Million |
| Milligrams Per Gram | × | $10^{3}$ | = Parts Per Million |
| Cubic Millimeters Per Liter | × | 1.0 | = Parts Per Million (Volume) |
| Cubic Microns Per Milliliter | × | $10^{-6}$ | = Parts Per Million (Volume) |
| Parts Per Million | × | 8.345 | = Pounds Per Million Gallons |

## POUNDS

| | | |
|---|---|---|
| Milligrams Per Square Meter | $\times$ 8922 | = Pounds Per Acre |
| Grams Per Square Meter | $\times$ 8.922 | = Pounds Per Acre |
| Kilograms Per Hectare | $\times$ 0.8922 | = Pounds Per Acre |
| Milligrams Per Square Centimeter | $\times$ 89.22 | = Pounds Per Acre |
| Milligrams Per Liter | $\times$ 2.72 | = Pounds Per Acre-foot |
| Milligrams Per Cubic Meter | $\times$ 2.72 $\times$ $10^{-3}$ | = Pounds Per Acre-foot |
| Micrograms Per Square Meter | $\times$ 8.92 $\times$ $10^{6}$ | = Pounds Per Acre |
| Acre-feet of Water | $\times$ 2.7 $\times$ $10^{6}$ | = Pounds of Water |
| Gallons of Water | $\times$ 8.345 | = Pounds of Water |

Parts Per Million $\times$ Cubic Feet Per Second $\times$ 5.4 = Pounds Per Day
(Gallons Per Minute $\times$ 2.228 $\times$ $10^{-3}$ = Cubic Feet Per Second)

Parts Per Million $\times$ 8.34 $\times$ Gallons Per Day $\times$ $10^{-6}$ = Pounds Per Day
(Gallons Per Minute $\times$ 1440 = Gallons Per Day)

## STANDARD UNITS

Areal Standard Units = $20\mu \times 20\mu = 400\mu^2$

Cubic Standard Units = $20\mu \times 20\mu \times 20\mu = 8000\mu^3$

Cubic Standard Units $\times$ 8 $\times$ $10^{-3}$ = Parts Per Million By Volume

## VOLUME

| | |
|---|---|
| Cone | —Square the radius of the base $\times$ 3.1416 $\times$ height $\div$ 3. |
| Cube | —Cube the length of one edge. |
| Cylinder | —Square the radius of the base $\times$ 3.1416 $\times$ height. |
| Pyramid | —Area of the base $\times$ height $\div$ 3. |
| Sphere | —Cube the radius $\times$ 3.1416 $\times$ 4 $\div$ 3. |

# PLANKTON COUNTING

Some waters contain (phyto- and/or zoo-plankton) in numbers large or small as to require dilution or concentration of samples as the case may be, to obtain accurate counts. Many natural waters require neither dilution nor concentration and should be enumerated directly. Correspondingly, zooplankton usually are not adequately abundant to be accurately counted and

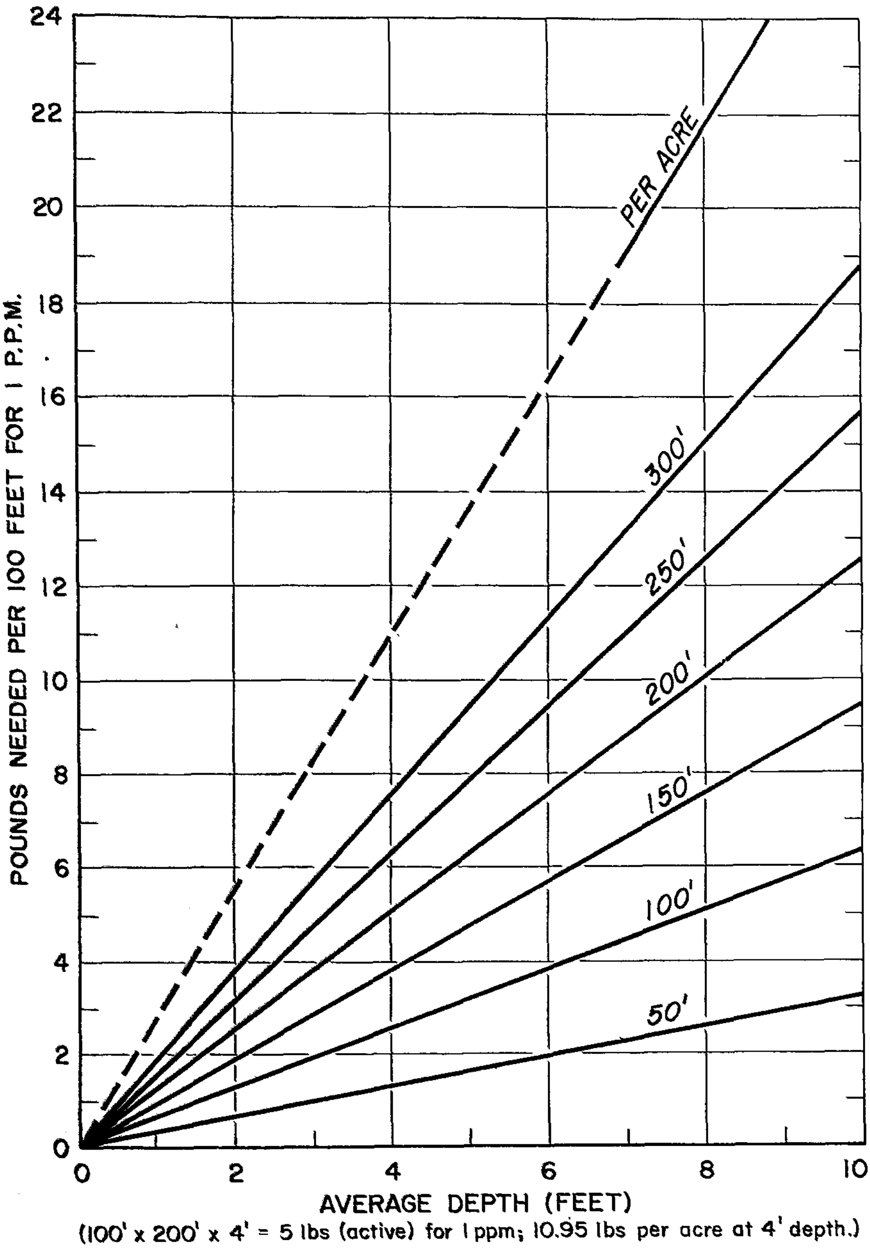


**Figure 1. Chemical Dosage Chart. To achieve a chemical concentration of 1 mg/1 in water having an average depth of 8 feet requires 10 pounds of the active chemical for an area 200 feet by 100 feet, or 21.8 pounds per acre.**

require concentration. Selection of methods and materials used in plankton enumeration depends on the study objectives, density of plankters in the waters being investigated, equipment available, and the investigator's experience.

## A. SEDGWICK-RAFTER COUNT

The Sedgwick-Rafter (S-R) cell has been and continues to be the most commonly employed device for plankton enumeration. It is easily manipulated and provides reasonably reproducible information when used with a calibrated microscope equipped with an eyepiece measuring device, usually a Whipple micrometer. It can be used to enumerate undiluted, concentrated, or diluted plankton samples. The biggest disadvantage associated with the S-R cell is magnification. The S-R cell cannot be used to enumerate very small plankton unless the microscope is equipped with special lenses that provide sufficient magnification (400x or greater) and clearance between objective lenses and the S-R cell.

The Sedgwick-Rafter cell is 50 mm long by 20 mm wide by 1 mm deep. Since the total area is 1,000 $mm^2$, the total volume is $1 \times 10^{12}$ cubic $\mu$, 1,000 $mm^3$, or 1 ml. A "strip" the length of the cell thus constitutes a volume 50 mm long, 1 mm deep, and the width of the Whipple field. Two or four strips usually are counted, depending on the density of plankters. Counting more than four strips is not expedient when there are a lot of samples to be enumerated; concentrating procedures then should be employed, and counts made of plankters in the concentrate.

$$\text{No. per ml} = \text{Actual Count} \times \frac{1{,}000}{\text{Volume of ``strip'' } (mm^3)}$$

If the sample has been concentrated, the concentration factor is divided into the actual count to derive the number of organisms per ml. For separate field counts (usually 10 or more fields):

$$\text{No. per ml} = \text{ave. count per field} \times \frac{1{,}000}{\text{Volume of field} \times \text{No. of fields}}$$

When special lenses are not used and there is a need to enumerate small plankton, usually very abundant, certain other procedures outlined below may be employed in conjunction with and related to counts obtained from the S-R cell.

## B. MEMBRANE FILTER

(McNabb, C. D. 1960. Enumeration of Freshwater Phytoplankton Concentrated on the Membrane Filter. Limnology and Oceanography, *5*(1) : 57-61.

The membrane filter method of plankton counting requires a vacuum pump, special filtering papers, and experience in determining the proper amount of sample to be filtered. Plankton in water samples containing substantial quantities of suspended matter such as silt may be difficult to enumerate by this method since in the process of filtering the suspended matter tends to crush the plankton or otherwise obscure them from the investigator's view. However, the method has certain features that make it particularly adaptable for use on most other waters. Primary among these features, the method permits the use of conventional microscope lenses to achieve high magnification for enumeration of small plankton (the membrane filter retains very small organisms), provides relatively rapid processing of samples if the investigator is familiar with the procedure and the plankton, does not require counting of individual plankters to derive enumeration data, and increases the probability of observing the less abundant forms.

The sample is filtered through a 1-inch membrane filter. The wet filter is removed and placed on top of two drops of immersion oil on a microscopic slide; two drops of immersion oil are placed on top of the filter. The filter is air-dried at room temperature until clear (approximately 48 hours). A cover slip is added prior to examination.

When examined, the magnification and sampling field or quadrat must be of such size that the most abundant species will appear in at least 70 but not more than 90 percent of the microscopic quadrats examined (80 percent is optimum). Otherwise the field size or the amount of sample concentrated must be altered. The occurrence of each species in 30 random microscopic fields is recorded.

Number of organisms per milliliter = density (d) from following table × number of quadrats or fields on membrane filter ÷ number of milliliters filtered × formalin dilution factor [0.96 for 4 percent formalin].

**CONVERSION TABLE FOR MEMBRANE FILTER TECHNIQUE**
**(Based on 30 Scored Fields)**

| TOTAL OCCURRENCE | F% | D |
|---|---|---|
| 1 | 3.3 | 0.03 |
| 2 | 6.7 | 0.07 |
| 3 | 10.0 | 0.10 |
| 4 | 13.3 | 0.14 |
| 5 | 16.7 | 0.18 |
| 6 | 20.0 | 0.22 |
| 7 | 23.3 | 0.26 |
| 8 | 26.7 | 0.31 |
| 9 | 30.0 | 0.35 |
| 10 | 33.3 | 0.40 |
| 11 | 36.7 | 0.45 |
| 12 | 40.0 | 0.51 |
| 13 | 43.3 | 0.57 |
| 14 | 46.7 | 0.63 |
| 15 | 50.0 | 0.69 |
| 16 | 53.3 | 0.76 |
| 17 | 56.7 | 0.83 |
| 18 | 60.0 | 0.91 |
| 19 | 63.3 | 1.00 |
| 20 | 66.7 | 1.10 |
| 21 | 70.0 | 1.20 |
| 22 | 73.3 | 1.32 |
| 23 | 76.7 | 1.47 |
| 24 | 80.0 | 1.61 |
| 25 | 83.3 | 1.79 |
| 26 | 86.7 | 2.02 |
| 27 | 90.0 | 2.30 |
| 28 | 93.3 | 2.71 |
| 29 | 96.7 | 3.42 |
| 30 | 100.0 | ? |

$$\text{Where F} = \frac{\text{total number of species occurrences}}{\text{total number of quadrats examined}} \times 100$$

## C. DROP COUNT

(Prescott, G. W. 1951. The Ecology of Panama Canal Algae. American Miscroscopical Society, *70:* 1-24).

Plankton samples that have been highly concentrated can be enumerated by the drop count method. It requires only basic equipment such as microscope, slides, cover slips, and a calibrated large-pore dropper, and facilitates use of high power lenses for identification and enumeration of organisms. Certain disadvantages are inherent in the method: 1) because water normally is used as a mounting medium enumeration must be accomplished relatively rapidly to prevent dessication and subsequent distortion of organisms; 2) results are not sufficiently accurate when only one slide-mount is examined, thus necessitating preparation and enumeration of at least three or more slide-mounts; and 3) the investigator should be sufficiently familiar with plankton to rapidly identify and count the specimens encountered. The concentrate is thoroughly mixed in the stored vial. A large-pore dropper delivering a known number of drops per milliliter is used to transfer one drop of concentrate to a glass slide. A cover slip is applied; 5 low-power fields and 10 high-power fields are examined, and the number of each species is recorded at the magnifications used. Enumeration is repeated on 3 such mounts for a total of 15 low-power fields and 30 high-power fields.

No. per ml = ave. no. per field × no. of fields per drop or per cover slip × no. of drops per ml ÷ the concentration factor.

The concentration factor = ml of original sample ÷ ml of concentrate × (100—percent of preservative in sample).

## D. DIATOM COUNTS

Some phytoplankton samples primarily consist of diatoms. Such organisms generally are difficult to identify without special preparation as distinguishing characteristics are obscured by protoplasm. Destruction of the protoplasm by heat or chemicals provides recognition of taxonomic features. Destruction by heat often is preferred to that by chemicals because the former does not require special glassware and reagents, reduces the risk of losing organisms during sample preparation, and decreases processing time. When there is need to derive diatom data, such organisms can be properly concentrated by settling-decanting or centrifuge-decanting techniques that employ a 2 to 4 percent solution of household detergent to free organisms lodged on the walls of sample containers and water-surface films.

A cover slip is placed on a hot plate that is sufficiently warm to increase the evaporation rate of (but not boil) the concentrated plankton sample. Several drops of concentrate are transferred to the cover slip by means of a large-pore calibrated dropper and allowed to evaporate to dryness. (This may be repeated on concentrates containing few diatoms until the entire sample has been transferred to the cover slip, but precautions are taken to prevent a residue that is too dense to recognize the organisms.) After evaporation, the residue on the cover slip is incinerated on the hot plate at temperatures ranging from 600-1,000°F, effecting adequate incineration in $3/4$ to $1/3$ hour respectively. A drop of distilled water is placed on a clean slide. The cooled cover slip with its residue is carefully transferred to the slide thus forming a water mount for identification and enumeration of diatoms. Permanent and more easily handled mounts, especially for processing at high-dry and oil immersion magnifications, are prepared by using Hyrax instead of water as a mounting medium. When Hyrax is used, heating of the slide to near 200°F for 1 to 2 minutes prior to application of the cover slip hastens evaporation of solvent in the Hyrax and reduces curing of the medium to about 20 seconds (solvent-free Hyrax is hard and brittle at room temperature). A firm but gentle pressure is applied to the cover glass by means of a forceps or other suitable instrument during cooling of the Hyrax mount (about one minute) to assure penetration of the medium into the diatom cells.

Enumeration and calculation to derive numbers of diatoms per ml are similar to those for the drop count. If examination reveals uneven distribution of diatoms in either the water or Hyrax mount, only proportionate counts of the species present are conducted and these are related to enumerations made by previously outlined methods.

## E. ALGAL VOLUME

Expression of plankton data as numbers of individuals per unit of water is often not meaningful since such data are only indices of the amount of plankton present. Similarly, plankton data derived and reported as *areal standard units* or *cubic standard units* are somewhat obscure because such units are arbitrarily selected and do not directly connote the amount of plankton present on a volume to volume or weight to weight basis: the former unit is a square surface with edges of 20 microns ($\mu$); the latter is a cube 20 $\mu$ long, 20 $\mu$ wide, and 20 $\mu$ deep.

Plankton data derived and reported on a volume to volume basis (ppm) are more useful and more widely understood than other data. Optical measurements with a calibrated microscope and ocular micrometer are best suited to other plankton. The shape of such organisms determines the measurements made to derive their volume, and the unit of measurement is the micron.

Wet Algal Volume (ppm) = Number of organisms per milliliter × average species volume in cubic microns × $10^{-6}$.

### BIO-ASSAY DILUTION CHART

A Guide to the Selection of Experimental Concentrations, Based on Progressive Bisection of Intervals on a Logarithonic Scale

| Col. 1 | Col. 2 | Col. 3 | Col. 4 | Col. 5 |
|---|---|---|---|---|
| 10.0 | | | | |
| | | | | 8.7 |
| | | | 7.5 | |
| | | | | 6.5 |
| | | 5.6 | | |
| | | | | 4.9 |
| | | | 4.2 | |
| | | | | 3.7 |
| | 3.2 | | | |
| | | | | 2.8 |
| | | | 2.4 | |
| | | | | 2.1 |
| | | 1.8 | | |
| | | | | 1.55 |
| | | | 1.35 | |
| | | | | 1.15 |
| 1.0 | | | | |

### BIO-ASSY DOSAGE CHART

For Preliminary Screening of an Effluent Waste

| Percent of waste in test jar | Waste added (ml) | Dilution water added (ml) |
|---|---|---|
| 100 | 2,500 | 0 |
| 75 | 1,875 | 625 |
| 56 | 1,400 | 1,100 |
| 32 | 800 | 1,700 |
| 18 | 450 | 2,050 |
| 5.6 | 140 | 2,360 |
| 1 | 25 | 2,475 |
| 0 | 0 | 2,500 |
| Total (ml) | 7,190 | 12,810 |
| Gallons required | 2 | 3 1/3 |

# DILUTION TABLE

*Concentration Desired*

To prepare solutions of concentrations indicated at left, take number of milliliters of stock solution shown below, and make up to one liter with suitable dilution water.

| % | ppm or mg/L | ppb or µg/L | Stock sol: 10 % 100 gm/L | Stock sol: 1 % 10 gm/L | Stock sol: .1 % 1 gm/L | Stock sol: .01 % 1 gm/L | Stock sol: .001 % .01 gm/L |
|---|---|---|---|---|---|---|---|
| 100. | 1,000,000 | | | | | | |
| 10. | 100,000 | | 1,000 | | | | |
| 5.6 | 56,000 | | 560 | | | | |
| 3.2 | 32,000 | | 320 | | | | |
| 1.8 | 18,000 | | 180 | | | | |
| 1.0 | 10,000 | | 100 | 1,000 | | | |
| .56 | 5,600 | | 56 | 560 | | | |
| .32 | 3,200 | | 32 | 320 | | | |
| .18 | 1,800 | | 18 | 180 | | | |
| .1 | 1,000 | | 10 | 100 | 1,000 | | |
| .056 | 560 | | 5.6 | 56 | 560 | | |
| .032 | 320 | | 3.2 | 32 | 320 | | |
| .018 | 180 | | 1.8 | 18 | 180 | | |
| .01 | 100 | | 1.0 | 10 | 100 | 1,000 | |
| .0056 | 56 | | | 5.6 | 56 | 560 | |
| .0032 | 32 | | | 3.2 | 32 | 320 | |
| .0018 | 18 | | | 1.8 | 18 | 180 | |
| .001 | 10 | | | 1.0 | 10 | 100 | 1,000 |
| .00056 | 5.6 | | | | 5.6 | 56 | 560 |
| .00032 | 3.2 | | | | 3.2 | 32 | 320 |
| .00018 | 1.8 | | | | 1.8 | 18 | 180 |
| .0001 | 1.0 | 1,000 | | | 1.0 | 10 | 100 |
| .000056 | .56 | 560 | | | | 5.6 | 56 |
| .000032 | .32 | 320 | | | | 3.2 | 32 |
| .000018 | .18 | 180 | | | | 1.8 | 18 |
| .00001 | .10 | 100 | | | | 1.0 | 10 |
| .0000056 | .056 | 56 | | | | | 5.6 |
| .0000032 | .032 | 32 | | | | | 3.2 |
| .0000018 | .018 | 18 | | | | | 1.8 |
| .000001 | .010 | 10 | | | | | 1.0 |

## BIO-ASSAY SHEET

Name of Material Tested: ______________________________

Source of Material: ______________________________

Test Animal(s): ______________________________

Date: ______________ Time Begun: ______________ Quantity of Dilution Water: ______________

Strength of Test Solution: ______________ Air Added: ______________ Water Temp: ______________

| Aquaria No. | cc. of test solu. added | Concentration p.p.m. | No. of Test Animals | 24-hour Survival | 24-hour % | 48-hour Survival | 48-hour % | 72-hour Survival | 72-hour % | 96-hour Survival | 96-hour % | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |
| 0 | | | | | | | | | | | | |

Concentration (p.p.m.) at which 50% of test animals survive: $TL_m$

Using Doudoroff's (S. & I.W. 23 (11) 1380–1397) proposed tentative formula for the estimation of a presumably biologically safe concentration (C):

$$C = \frac{48\text{-hr. } TL_m \times 0.3}{\left(\frac{24\text{-hr. } TL_m}{48\text{-hr. } TL_m}\right)^2} = \_\_\_\_\_\_\_\_ =$$

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