



Biota and Biological Parameters as Environmental Indicators

Biota and Biological Parameters as Environmental Indicators

Edited by Phillip E. Greeson

B r i e f i n g P a p e r s o n W a t e r Q u a l i t y

G E O L O G I C A L S U R V E Y C I R C U L A R 8 4 8 - B

United States Department of the Interior

JAMES G. WATT, *Secretary*



Geological Survey

Doyle G. Frederick, *Acting Director*

Library of Congress Cataloging in Publication Data

Main entry under title:

Biota and biological parameters as environmental indicators.

(Briefing papers on water quality) (Geological Survey circular ; 848-B)

Bibliography: p.

Supt. of Docs. no.: I 19.472:848-A

1. Water quality bioassay. I. Greeson, Phillip E. II. Series. III. Series: Geological Survey circular ; 848-B.
QE75.C5 no. 848-B [QH96.8.B5] 557.3s 81-607891 [628.1'61] AACR2

*Free on application to Distribution Branch, Text Products Section,
U. S. Geological Survey, 604 South Pickett Street, Alexandria, VA 22304*

FOREWORD

In August 1974, the Water Resources Division of the U.S. Geological Survey introduced the first of a series of briefing papers that were designed to increase the understanding of its employees of the significance of various aspects of water quality. Numerous briefing papers have been prepared by the Quality of Water Branch. Others will be prepared as the need arises. Each paper addresses a separate topic and is written in a nontechnical, easy-to-understand manner for distribution within the organization.

Because of the favorable reception that the papers have received and their apparent effectiveness in accomplishing the objective stated above, it would appear that their wider distribution would serve a useful purpose. It is hoped that a wide range of persons, including those interested in the quality of our Nation's water resources, but who have little or no technical training, will find value in reading the papers. Furthermore, it is hoped that the papers will be suitable for supplemental reading in secondary education programs and in beginning college-level courses.

The U.S. Geological Survey plans to publish several U.S. Geological Survey Circulars that contain compilations of briefing papers on particular aspects of water quality. This second Circular contains four papers describing selected biota and biological parameters as environmental indicators.

Philip Cohen
Chief Hydrologist

CONTENTS

Foreword	Page III
Abstract	B1
Species diversity and its measurement, by Robert C. Averett	3
Algal growth potential, by W. Thomas Shoaf	7
Estimation of microbial biomass by measurement of adenosine triphosphate (ATP), by W. Thomas Shoaf	11
Lake classification – Is there method to this madness?, by Dennis A. Wentz	15

ILLUSTRATIONS

FIGURE 1. Characteristic growth curves of a unicellular alga in a culture of limited volume ____	Page B7
2. Summary of the major trophic types of lakes	18
3. Hypothetical curve of the course of natural eutrophication of lakes	18
4. Effect of lake depth on the process of eutrophication	18

BRIEFING PAPERS ON WATER QUALITY

**Biota and Biological Parameters
as Environmental Indicators**

Edited by Philip E. Greeson

ABSTRACT

This is the second of several compilations of briefing papers on water quality prepared by the U.S. Geological Survey. Each briefing paper is prepared in a simple, nontechnical, easy-to-understand manner. This U.S. Geological Survey Circular con-

tains papers on selected biota and biological parameters as environmental indicators. Briefing papers are included on "Species diversity and its measurement," "Algal growth potential," "Estimation of microbial biomass by measurement of adenosine triphosphate (ATP)," and "Lake classification—Is there method to this madness?"

Species Diversity and its Measurement

By Robert C. Averett

ABSTRACT

With the decrease in popularity of the single-species indicator-organism concept, there arose an understanding in the natural diversity of organisms. This, combined with the science of information theory, led to the development of formulae for computing a diversity index. Interpretation of an index value has limitations; but, when used properly, it is a valuable tool for the assessment of water quality.

INTRODUCTION

Man has always marveled at the number of different types of organisms on Earth. Sketches on cave walls are testimony that early man not only knew about many types of animals, but also knew something about their roles in nature. Today we recognize about 37,000 species of micro-organisms, 341,000 species of plants, and 1,358,000 species of animals. Taxonomists (scientists that classify organisms) continually are adding to these numbers as new organisms are discovered. The insects are the largest single group of organisms and have been only partially classified. It is estimated that if all the different kinds of insects on Earth were classified at this time, they would exceed 2 million species—a figure that exceeds the known types of all other animals.

The biological definition of a species is a group of actually or potentially interbreeding organisms reproductively isolated from other such groups of organisms. A true species must meet this criterion. It is this concept of a species that man uses in his husbandry of domestic animals, and in his use of organisms in defining a particular environment.

CONCEPT OF SINGLE-SPECIES INDICATORS

The use of organisms to indicate certain environmental conditions stemmed from the work of botanists of the 18th and 19th centuries. Plants, because they responded to temperature and other climatic variables, as well as to soil types, were used as an aid in the classification of the physical

environment. In a similar manner, the presence or absence of specific types of animals was used to delineate certain climatic conditions. The use of these biological indices became more popular as more knowledge was gained about the distribution and abundance of organisms.

The first comprehensive work on using aquatic organisms to define water-quality conditions was by two German biologists, Kolkwitz and Marsson (1908, 1909). They published findings on a stream that received domestic wastewater. From the standpoint of organism types and diversity, they were able to divide the stream into four distinct reaches, which they called zones: the clean water zone, the septic zone where the sewage is first introduced, the zone of active sewage decomposition, and the zone of recovery.

Other workers followed this classification procedure, or modified it slightly to fit their own findings. Each zone of the stream was inhabited by distinct groups of organisms. Moreover, in those zones where the waste load was heaviest and where the stream bottom was covered by waste solids, the number of organisms per unit area was very high, but the number of different kinds of organisms was very low. In other words, there were many organisms, but the diversity (number of species) was low. Further, nymphs and larvae of some stream insects (particularly the mayflies, stoneflies, and caddisflies) were frequently absent from areas receiving heavy loads of organic waste but were present in clean water reaches. Much of this absence resulted from the alteration of the physical stream bottom by waste sludge. In addition, low oxygen concentrations and an altered food supply prevented them from living in the heavily polluted section of the stream. In contrast, some trueflies (for example, midges, gnats), that were adapted to living in the sludge of a stream bottom and in water that had a low oxygen concentration, thrived, and in the absence of competition,

their numbers per unit areas was very high. These findings led to the single-species indicator-organism concept whereby specific groups of organisms were unadapted to pollution, but others were able to tolerate significant changes in the environment.

The single-species indicator-organism concept had several failings. First, it was based only upon organic (oxygen-demanding) waste loadings. Sublethal levels of toxic materials (some industrial and agricultural wastes) were ignored, yet they also may be preferential as to which organisms they kill. All organisms, including man, tolerate varying concentrations of potentially toxic materials, and a concentration that is toxic to one organism, or a group of physiologically similar organisms, may not be toxic to others. Consequently, there may be great variability in the effects of toxic materials on the distribution and abundance of aquatic life.

The second failing was that organisms that make up the groups found in the various stream reaches have had to come from somewhere. That is, they are all natural members of the stream fauna, but, when the stream is altered, some find the habitat more suitable and their numbers increase. Those that find the habitat less suitable die or leave the area; for example, the presence of truefly larvae in a sample does not indicate pollution or water-quality degradation. The single-species indicator-organism concept, therefore, is not sound unless practiced by someone thoroughly knowledgeable in the habitat requirement and physiology of the organisms. As a general biological technique for assessing water quality, it has severe limitations.

CONCEPT OF SPECIES DIVERSITY

At about the time that the single-species indicator-organism concept was losing its popularity (not because it was completely erroneous, but because it was too often misused), the species-diversity concept was becoming popular. This concept considered the entire community of aquatic organisms and, thus, was sound from a biological standpoint. Initially, the concept consisted of simply counting the number and types of organisms in a given sample. Later, the ratio of the number of organisms to the number of species was used as a simplified species-diversity index. While this technique also provided a quantitative approach, it was difficult to interpret the results because the ratios often changed greatly as the number of organisms in the sample increase.

Later, the science of information theory was applied to biological sampling problems. Briefly, the

objective of information theory is to measure the amount of order or disorder in a system. In the biological community, we may wish to measure a number of things, but for our purposes the most important would be the number of species and the number of individuals in each species. That is, we may want to look at the richness or variety of the community structure and ask the question, "How difficult (or easy) will it be to correctly predict the species of the next individual in the sample?" This is similar to a message receiver predicting the next symbol, for example, dot or dash, in a Morse code message. Because it is dot-dash, on-off, or yes-no, relations can be represented by 0-1 digits, or binary digits. They often are measured in terms of bits (for number of binary digits).

The information theory diversity index is calculated using the function:

$$\delta = - \sum_{i=1}^s p_i \log_2 p_i = - \sum (ni/n) \log_2 (ni/n) \quad (1)$$

where: δ = species diversity or information content of the sample (bits/individual).

The term δ often is denoted by H .

s = number of species in the sample

$p_i = ni/n$ = proportion of the total sample belonging to the i th species.

This function, developed simultaneously but independently, by Wiener (1948) and Shannon and Weaver (1949), is a measure of the amount of uncertainty. The larger the amount of uncertainty and, hence, the greater the diversity of the species, the greater the diversity or the value of δ . For example, a message such as $\alpha\alpha\alpha\alpha$ has no uncertainty, and, thus, no diversity and, therefore, $\delta=0$. In a sample where all organisms are of the same species (a rare event in nature), the diversity would be zero. In other words, in a single-species sample, one individual would tell as much about the whole sample as would any other individual, or all of the individuals combined. In a sample of many species, we would have to identify more organisms, if we wished to exhaust all of the information from the sample. Thus, a sample in which each organism belonged to a different species (again, a rare event in nature), would have a high diversity and δ would be a positive number. Refer again to our example where the message $\alpha\alpha\alpha\alpha$ has no uncertainty. In contrast, the message $\beta\alpha\Phi\gamma$ has a high uncertainty, and predicting the next letter would be much more difficult, and δ would be some positive number. Note that logarithms to the base 2 are used so as to obtain bits per individual.

The diversity index, δ , is influenced by the number of species in the sample as well as by the

evenness of the distribution of the species in the sample. The measure of evenness is attained first by calculating the maximum diversity, $\delta \max$, as:

$$\delta \max = - \sum_{i=1}^s \left(\frac{1}{s} \log_2 \frac{1}{s} \right) = \log_2 s \quad (2)$$

The highest value of $\delta \max$ is attained when the number of individuals per species is distributed equally—that is, when all the species are present in the same proportion $\frac{1}{s}$. In two samples in which the species are distributed evenly, the sample with the largest number of species will have the greatest diversity (Pielou, 1969). From equations 1 and 2, a measure of the evenness of the species in the sample (J) can be calculated as:

$$J = \frac{\delta}{\delta \max} \quad (3)$$

The diversity index, δ , has obvious advantages over the single-species indicator-organism concept, or the simple method of counting the number of species in a sample. First, it is a dimensionless equation, and numbers or biomass (weight per volume or area) can be used. Second, the importance of each species in the community is expressed as a ratio representing the contribution of each species to the total diversity or biomass. Third, and of great importance, diversity indices derived from information theory are virtually independent of sample size. The occurrence of rare species, as additional samples are collected, does not greatly change the value of δ .

In a group of 10 samples from four habitats (one of which was a sample board), Wilhm and Dorris (1968) found that the diversity reached 95 percent of the asymptotic diversity (that value of δ that essentially does not change with the addition of new samples) before five samples were collected. In a subsequent paper, Wilhm (1970) reported that the diversity index ranged from 0 to 10, but usually was less than 5. In streams that do not receive waste material (clean water zones), it was between 3 and 4, while, in streams or stream reaches receiving organic waste, the diversity-index was usually less than 1. Wilhm (1970) further suggested that the asymptotic diversity value should be used. Generally, as mentioned, this occurs between two and five samples.

LIMITATIONS OF DIVERSITY INDEX VALUES

While the diversity index has a number of positive attributes, it also has its limitations. It is not a panacea for the problem of measuring water-

quality changes. Rather, it is a tool, and, like a tool, it must be used correctly to be effective. There are precautions that must be observed. First, the level of taxonomic level above species is valid. While desirable, it is not necessary to classify all organisms to the species level. Second, the physical habitat, such as velocity of flow and stream bottom material must be considered and reported. Streams that have various sizes of bottom material will have a greater diversity of organisms than streams that have material all of a similar size. Third, the season of the year and life history of the organisms must be considered. Some organisms have annual periods of dominance—for example, immediately after egg hatching, a particular group of organisms may be abundant, but shortly thereafter, natural mortality factors take their toll and reduce the number of individuals. Fourth, the method of collection and the mesh size of the screen used to separate the organisms is of utmost importance. It is invalid to compare diversity indices where different collecting gear and screen sizes have been used. Consequently, comparisons of diversity indices must be between similar taxonomic categories, similar substrates, and at similar times of the year, using similar collecting and separating techniques.

Regardless of these limitations, the diversity index (d) is a valuable tool, and with it a more detailed quantitative assessment of water quality can be made. It does not, however, explain the role of the individual organism in the community, the physiological stress that water-quality changes place upon the organisms, or the physiological stress that the use of the water may place upon man. These processes will never be explained with something as simple as the diversity index. Happily, continued research is providing information on the habitat, behavioral, and physiological requirements of aquatic organisms and ultimately will lead to a greater understanding and use of species diversity and the diversity index as one tool for assessing water-quality changes.

REFERENCES

- Kolkwitz, R., and Marsson, M., 1908, *Ökologie der pflanzlichen Saprobien: Gerichte der Deutschen Botanischen Gesellschaft* 26a, p. 505-519. (Translated 1967, *Ecology of plant saprobia*, p. 47-52, in Keup, L. E., Ingram, W. M., and Mackenthun, K. M., eds., *Biology of water pollution: Federal Water Pollution Control Administration, U.S. Department of the Interior*, 290 p.).
- , 1909, *Ökologie der tierischen Saprobien: Beiträge zur Lehre von der biologischen Gewässerbeurteilung. Internationale Revue der Gesamten Hydrobiologie und*

- Hydrogeographie 2, p. 126-152. (Translated 1967, Ecology of animal saprobia, p. 85-95, in Keup, L. E., Ingram, W. M., and Mackenthun, K. M., eds., Biology of water pollution: Federal Water Pollution Control Administration, U.S. Department of the Interior, 290 p.).
- Margalef, Ramon, 1968, Perspectives in ecological theory: Chicago, Ill., University of Chicago Press, 111 p.
- Pielou, E. C., 1969, An introduction to mathematical ecology: New York, John Wiley and Sons, 268 p.
- Shannon, C. E., and Weaver, Warren, 1949, The mathematical theory of communication: Urbana, Ill., University of Illinois Press, 177 p.
- Warren, C. E., 1971, Biology and water pollution control: Philadelphia, Pa., W. B. Saunders Co., 434 p.
- Wiener, Norbert, 1948, Cybernetics: Cambridge, Massachusetts Institute of Technology Press, 194 p.
- Wilhm, J. L., 1970, Range of diversity index in benthic macro-invertebrate populations: Journal of the Water Pollution Control Federation, v. 42, no. 5, pt. 2, p. R221, R224.
- Wilhm, J. L., and Dorris, T. C., 1968, Biological parameters for water quality criteria: Bioscience, v. 18, no. 6, p. 477-481.

Algal Growth Potential

By W. Thomas Shoaf

ABSTRACT

Algal growth potential (AGP) is defined as the maximum algal mass that can be produced in a natural water sample under standardized laboratory conditions. Measurements of AGP can be used for a variety of purposes; however, an understanding of the principles of the test and the factors that affect its expression is critical to proper data interpretation.

DEFINITION

Algal growth potential (AGP) is defined as the maximum algal mass (dry weight) that can be produced in a natural water sample under standardized laboratory conditions. The characteristic growth pattern for unicellular algae in a culture of limited volume is shown in figure 1.

AGP is the algal mass present at stationary phase and is expressed as milligrams per liter dry weight of algae produced. Dry weight was chosen as a measure of algal growth because it is the most reliable parameter for all sample sources.

STANDARDIZATION OF ANALYSIS

Determination of the AGP of a water sample using the natural algae present is perhaps most

desirable. The presence of these algae and associated micro-organisms suggests that they are suited to survive the environment in which they live, and their potential for accelerated growth is of primary interest in many situations. The primary difficulty in using indigenous algae is the time required for measuring growth of diverse forms of algae such as colonial, filamentous, or univellular forms. In some instances, water samples contain few algae, and the introduction of foreign algae would be needed to insure a growth response.

The AGP of a number of samples can be compared only when variables which affect algal growth, such as light and temperature, are standardized and controlled. The need for standardization of techniques for measuring AGP for routine analysis was recognized by the Joint Industry/Government Task Force on Eutrophication (Bartsch, 1969). A cooperative developmental effort by the Soap and Detergent Association and the U.S. Environmental Protection Agency to produce a test for determining AGP in natural water samples was the result (Bartsch, 1971). *Selenastrum capricornutum* was selected as a test organism for the following reasons:

1. It is a unicellular alga and its growth can be monitored accurately and rapidly with an electronic particle counter.
2. *Selenastrum* has been shown to tolerate acidic and alkaline waters as well as oligotrophic (nutrient-poor water) and eutrophic (nutrient-rich water) conditions in aquatic environments (Forsberg, 1972).
3. It is listed among the most pollution-tolerant algae by Palmer (1969).
4. The AGP determined with *S. capricornutum* and the potential determined with the species indigenous to the water sample correlate well (Maloney and others, 1972).
5. The AGP for various freshwater environments using *S. capricornutum*, as measured by the

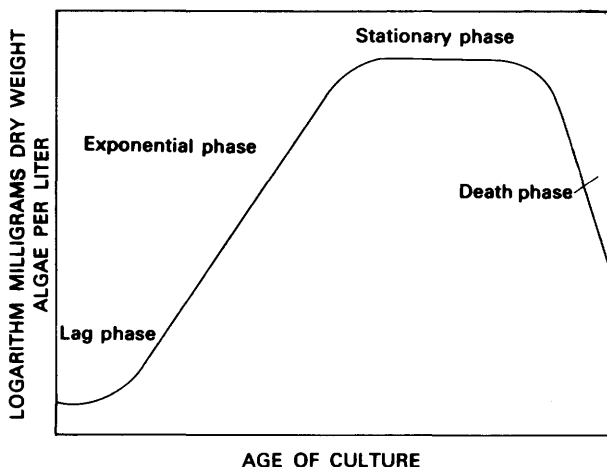


FIGURE 1.—Characteristic growth curve of a unicellular alga in a culture of limited volume.

standard test (Bartsch, 1971), varies widely from 0.1 to 248 milligrams dry weight of algae per liter (National Lake Survey Program, 1973).

APPLICATION

The design of a project is developed to meet a specific objective. All pertinent factors must be considered in planning if valid results and conclusions are to be obtained. It should be possible to evaluate AGP data more effectively in light of other biological, chemical, and physical measurements obtained by sampling at common sites. Various collection methods for streams and lakes have been described by Guy and Norman (1970), Greeson and others (1977), and Greeson (1979).

The objectives of AGP tests might include the establishment of baseline data or determination of the growth-controlling nutrient(s), the influence of a nutrient on algal growth, or the source of a nutrient when several inflows are involved. One of the first items that should be determined when measuring AGP is an understanding of what constitutes a significant change. A certain degree of variability can be expected with any assay or survey; however, once the baseline data are established, experimentation can be conducted.

The principle of the AGP determination is based on Liebig's Law of the Minimum, which recognizes that the development of a population is essentially regulated by the substance occurring in minimal quantity relative to the requirements of the population. AGP measurements have been used frequently to derive information on the required nutrients which are limiting algal growth (Leischman and others, 1979). This type of measurement usually is designed to examine in detail only a few nutrients which preliminary testing indicates may be limiting or in short supply. The approach is to observe the effect that certain growth promoting substances (nutrients) have upon the algae. These substances may be of precise known composition (Bartsch, 1971; Miller and Maloney, 1971; Maloney and others, 1972) or their composition may be somewhat less known, such as that of an effluent which may flow into a lake or stream from a suspected nutrient source (Brown and others, 1969). The practice of adding such substances to a water sample is called spiking. In the case of chemical spikes, it is obvious that if increasing amounts of a limiting nutrient are added to a water sample, eventually another factor becomes

limiting. The determination of how much and what to spike may be made easier when the concentration of the nutrient(s) of interest is known. Because algal growth in natural water samples can be limited by one or several nutrients in close succession, it might be necessary to test spikes of combinations of nutrients that could be limiting.

When only one nutrient is limiting algal growth, it is likely that a correlation will be observed between the concentration of that nutrient and the AGP. Some AGP studies have shown correlations between orthophosphate and algal growth (Wang and others, 1972), while correlations also are likely for other nutrients in other aquatic environments.

INTERPRETATION OF RESULTS

AGP data which are derived in the laboratory under controlled conditions of light and temperature do not necessarily reflect conditions in the natural aquatic environment from which the sample was taken. Only the potential at a given time can be measured. In nature, daily and seasonal variations in the intensity and duration of light occur, which can effect algal growth. Changes in other environmental factors can be important as well. For example, the rate of algal growth roughly doubles with every 11°C (20°F) rise in water temperature between 0°C (32°F) and 32°C (90°F) (Ferguson, 1968). Growth can be inhibited by any suspended material, living or nonliving, which interferes with the effective penetration of light essential to the algae. Grazing by invertebrates and fish, toxic materials entering the water, plant or animal excretions, or decay products from the algae themselves are all additional examples of factors that may inhibit growth potential.

In the standard procedure described by Bartsch (1971), Miller and others (1978), and Greeson (1979), membrane filtration of the water sample to remove indigenous algae, bacteria, fungi, and any other particles which might interfere with the procedure is required. These micro-organisms contain nutrients, which are not available to other algae while these organisms are living, but later become a source of nutrients as a result of decay after death. Thus, it is possible to measure a high concentration of algae during a "bloom," but observe a low AGP. After cell death and decay, the AGP probably will increase. This, as well as the other factors mentioned above, must be taken into consideration when attempting to make predictions about future algal growth.

SUMMARY

Algal growth potential is defined as the maximum algal mass (dry weight) that can be produced in a natural water sample under standardized laboratory conditions. AGP measurements are designed to establish baseline data, growth limiting factors (nutrients), and the influence and source of various growth promoting nutrients, so as to provide improved means for predicting and controlling excessive algal growth in aquatic habitats. Data can be compared only when the variables that control algal growth are standardized. Algal growth potentials derived in the laboratory may not reflect natural conditions because of insufficient light or temperature, grazing by invertebrates or fish, or the presence of toxic materials. An understanding of the principle of the test and the factors that affect the expression of AGP is critical to proper data interpretation.

REFERENCES CITED

- Bartsch, A. F., 1969, Provisional algal assay procedure: Corvallis, Oreg., U.S. Environmental Protection Agency, 61 p.
- 1971, Algal assay procedure: Bottle test: Corvallis, Oreg., U.S. Environmental Protection Agency, 82 p.
- Brown, R. L., Bain, R. C., and Tunzi, M. G., 1969, Effects of nitrogen removal on the algal growth potential of San Joaquin valley agricultural tile drainage effluents: Paper presented at the American Geophysical Union National Meeting, San Francisco, 15 p.
- Ferguson, F. A., 1968, A nonmyopic approach to the problem of excessive algal growth: Environmental Science and Technology, v. 2, p. 188-193.
- Forsberg, C. G., 1972, Algal assay procedure: Journal of the Water Pollution Control Federation, v. 44, p. 1623-1628.
- Greeson, P. E., ed., 1979, A supplement to—Methods for collection and analysis of aquatic biological and microbiological samples (U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4): U.S. Geological Survey Open-File Report 79-1279, 92 p.
- Greeson, P. E., Ehlke, T. A., Irwin, G. A., Lium, B. W., and Slack, K. V., eds., 1977, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A4, 332 p.
- Guy, H. P., and Norman, V. W., 1970, Field methods for the measurement of fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 3, Chapter C2, 59 p.
- Leischman, A. A., Greene, J. C., and Miller, W. E., 1979, Bibliography of literature pertaining to the Genus *Selenastrum*: Corvallis, U.S. Environmental Protection Agency, EPA-600/7-79-021, 191 p.
- Maloney, T. E., Miller, W. E., and Shiroyama, Tamotsu, 1972, Algal responses to nutrient addition in natural waters. I. Laboratory assays: Nutrients and eutrophication, Special Symposium, American Society of Limnology and Oceanography, v. 1, p. 155.
- Miller, W. E., and Maloney, T. E., 1971, Effects of secondary and tertiary wastewater effluents on algal growth in a lake-river system: Journal of the Water Pollution Control Federation, v. 43, p. 2361-2365.
- Miller, W. E., Green, J. C., and Shiroyama, Tamotsu, 1978, The *Selenastrum capricornutum* Printz algal assay bottle test—Experimental design, application, and data interpretation protocol: Corvallis, U.S. Environmental Protection Agency, EPA-600/9-78-018, 125 p.
- National Lake Survey Program, 1973, Highlights: Corvallis, Environmental Protection Agency, 5 p.
- Palmer, C. M., 1969, A composite rating of algae tolerating organic pollution: Journal of Phycology, v. 5, no. 1, p. 78-82.
- Wang, W. C., Sullivan, W. T., and Evans, R. L., 1972, A technique for evaluating algal growth potential in Illinois surface water: Urbana, Illinois State Water Survey, Report of Investigation 72, 16 p.

Estimation of Microbial Biomass by Measurement of Adenosine Triphosphate (ATP)

By W. Thomas Shoaf

ABSTRACT

Adenosine triphosphate is present in all living cells and is the primary energy donor in cellular processes. The measurement of the amount of adenosine triphosphate present in a water sample, therefore, is a measurement of the total biomass in the sample. The firefly luciferase reaction with luciferin is the commonly used method for determining the presence and amount of adenosine triphosphate in a sample. The method is very sensitive and rapid.

INTRODUCTION

For many ecological studies, it is important to know the total amount of living material or biomass present in an ecosystem. When dealing with larger animals and plants, it is relatively easy to measure biomass by conventional methods, such as collecting and weighing them. For the microorganisms, however, it can become very difficult. Both phytoplankton and zooplankton biomasses can be estimated by direct microscopic determination of volume and number. Bacteria can be estimated by various plating techniques (colony counting) and extinction dilution techniques. The techniques are often time-consuming, expensive, and subject to inherent sources of error. Some of the major problems include the following: Direct microscopic counting of bacteria may yield inaccurate biomass estimates because of the difficulty in distinguishing bacteria from bacteria-sized inert particles. There is, also, the inability to differentiate between living and nonliving cells, and the problems of cell aggregation characteristic of bacteria and algae. Low estimates may be produced by plating and extinction techniques which are selective because of the chemical composition of the media and variations in physical parameters such as temperature and pressure. The extinction dilution technique also may be biologically selective in that one form might overgrow the other.

For a total biomass determination, it would be desirable to measure some substance (for example,

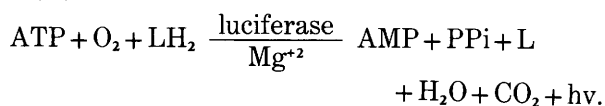
a biochemical compound) that is present in all living cells but is not associated with nonliving particulate material. This cellular substance should have a short survival time after the death of a cell, so that it would be specific for viable (living) biomass. It should be present in proportion to some measure of the total biomass for all microorganisms: algae, bacteria, fungi, and protozoans. Sensitive and accurate methods for its detection should be available.

The biochemical compound which seems to meet these requirements best is adenosine triphosphate (ATP). ATP is the primary energy donor in cellular life processes. Its central role in living cells and its biological and chemical stability make it an excellent indicator of the presence of living material. The level of endogenous ATP (that is, the amount of ATP per unit biomass) in bacteria (Allen, 1973), algae (Holm-Hansen, 1970), and zooplankton (Holm-Hansen, 1973) is relatively constant when compared to the cellular organic carbon content in several species of organisms. Furthermore, its concentration in all phases of a growth cycle remains relatively constant. In studies where cell viability was determined (Hamilton and Holm-Hansen, 1967; Dawes and Large, 1970), the concentration of ATP per viable cell remained relatively constant during periods of starvation. The quantity of ATP, therefore, can be used to estimate total living biomass.

ANALYTICAL DETERMINATION OF ATP

Very sensitive methods of ATP determination have been developed from the finding that luminescence in fireflies has an absolute requirement for ATP (McElroy, 1947). The ATP is determined by measuring the amount of light produced when ATP reacts with reduced luciferin (LH_2) and oxygen (O_2) in the presence of firefly enzyme luciferase and magnesium, producing adenosine

monophosphate (AMP), inorganic pyrophosphate (PPi), oxidized luciferin (L), water (H₂O), carbon dioxide (CO₂), and light (hv), by the following reaction:



The bioluminescent reaction is specific for ATP and the reaction rate is proportional to the ATP concentration; one photon of light is emitted for each molecule of ATP hydrolyzed. When ATP is introduced to suitably buffered (with respect to pH) enzymes and substrates (reduced luciferin and oxygen), a light flash follows; it decays in an exponential fashion. Either the peak height of the light flash or integration of the area under the decay curve can be used to form standard curves. Quantities of ATP as low as 10⁻¹² to 10⁻¹³ grams of ATP can be measured accurately.

APPLICATION

ATP analysis has been used to estimate the biomass at varying depths in the ocean (Holm-Hansen and Booth, 1966). The ATP levels found at various depths in the ocean indicate bacterial populations between 50 and 2,000 times those found by plating techniques. This may reflect the inadequacies of plating techniques referred to above.

The effect of chemical or thermal pollution on a system has been determined by passage of water through plant cooling systems and by monitoring the before and after levels of ATP. In such a study on the Chesapeake Bay, ATP appeared to be a reliable measure of biomass viability (Dupont Corporation, 1970). The effects of industrial plants and cities on streams can be evaluated in a similar fashion. Likewise, the effects of suspected (or known) biocides on micro-organisms also could be measured immediately, without the usual delay encountered with plate counts (for bacteria) or phytoplankton analysis (for algae).

Biomass determination by ATP measurement can be correlated with measurement of chlorophyll where the dominant life form is phytoplankton (Holm-Hansen, 1969). If bacteria or protozoans are present in large numbers and not evenly distributed with the phytoplankton, then a correlation with chlorophyll would not be expected (Holm-Hansen and Paerl, 1972). A ratio of ATP (biomass) to chlorophyll can be useful for determining whether the living matter in a sample is predominantly autotrophic (mainly algal) or contains a large number of heterotrophic organisms

(bacteria, protozoans). Although the ranges of values are yet unestablished, low values of the ATP to chlorophyll ratio should be indicative of autotrophic conditions, whereas high values imply a more heterotrophic community.

Since ATP can be correlated directly with living carbon (Holm-Hansen, 1973), another tool for accessing biological water quality is available in the ratio of living carbon (determined by ATP) to total organic carbon. This ratio may be a useful measure of the degree of eutrophication. In an oligotrophic situation, a high percentage of the total organic carbon is in living cells and, thus, the ratio of living carbon (determined by ATP) to total organic carbon will be at its highest. As conditions move toward the eutrophic, more nonliving organic carbon accumulates and the percent of living carbon (to the total) decreases. Determination of this ratio may, therefore, be a good method for describing the overall biological condition.

SUMMARY

The determination of biomass (total living material) has been a difficult task for investigators in the field of aquatic microbiology. Measurement of ATP provides a very sensitive and rapid estimate of biomass. While useful as an estimate of biomass alone, it also may be related to determinations of chlorophyll and total organic carbon to provide ratios which give more information about biological water quality.

REFERENCES CITED

- Allen, P. D., 1973, Development of the luminescence biometer for microbial detection: Developments in Industrial Microbiology, v. 14, p. 67-73.
- Dawes, E. A., and Large, P. J., 1970, Effect of starvation on the viability and cellular constituents of *Zymomonas anaerobia* and *Zymomonas mobilis*: Journal of General Microbiology, v. 60, p. 31-40.
- Dupont Corporation, 1970, Water ecology/waste water treatment: Wilmington, Del., Dupont Applications Brief no. 760BF1 (A72069), 2 p.
- Hamilton, R. D., and Holm-Hansen, O., 1967, Adenosine triphosphate content of marine bacteria: Limnology and Oceanography, v. 12, p. 319-324.
- Holm-Hansen, O., 1969, Determination of microbial biomass in ocean profiles: Limnology and Oceanography, v. 14, p. 740-747.
- 1970, ATP levels in algal cells as influenced by environmental conditions: Plant Cell Physiology, v. 11, p. 689-700.
- 1973, Determination of total microbial biomass by measurement of adenosine triphosphate, in Stevenson, L. H., and Colwell, R. R., eds., Estuarine microbial ecology: Columbia, University of South Carolina Press, p. 73-89.

- Holm-Hansen, O., and Booth, C. R., 1966, The measurement of adenosine triphosphate and its ecological significance: *Limnology and Oceanography*, v. 11, p. 510–519.
- Holm-Hansen, O., and Paerl, H. W., 1972, The applicability of ATP determination for estimation of microbial biomass and metabolic activity: *Memoirs of 1st Italia Idrobiologiae*, Supplement 29, p. 149–168.
- McElroy, W. D., 1947, The energy source for bioluminescence in an isolated system: *Proceeding of National Academy Sciences*, v. 33, p. 342–346.

Lake Classification—Is There Method to this Madness?

By Dennis A. Wentz

ABSTRACT

The number of scientific publications discussing lake classification schemes has increased considerably since the early 1970's. This has resulted primarily from the enactment of the Federal Water Pollution Control Act Amendments of 1972. This paper discusses lake classifications based upon geologic, hydrologic, and trophic schemes. Many of the schemes have limited applicability, while others are adaptable to a variety of situations.

INTRODUCTION

The study of lakes has developed into a popular hydrological pursuit in recent years, and there are those who would encourage even greater expansion in this area. Perhaps first, however, we would be well advised to pause and reflect on some of the more timely developments of current limnological research. One of these is in the area of lake classification.

The number of scientific publications addressing lake classification has increased considerably since the early 1970's. "To what end are these classifications dedicated?" you might ask. Or, "Why has there been a sudden interest in classifying lakes?" The purpose of this paper is to answer these questions and to provide some insight into the various lake-classification schemes that have been proposed. Although it has not been possible to review all lake classifications that have been suggested, the bibliographies included herein should provide an adequate base for anyone interested in further pursuing the topic. In particular, those trophic classifications based on the nutrient loading concepts introduced by Vollenweider (1968) have been specifically excluded, as their consideration would have made the paper excessively long.

Perhaps the first order of business is to define exactly what is meant by the term classification. According to Sokal (1974), classification is " * * * the ordering or arrangement of objects into groups or sets on the basis of their relationships."

(It should be pointed out that the relationships sometimes are presumed.) Thus, classification is the process of defining the groups into which objects are placed. The process of assigning an object to a previously defined group, on the other hand, can be described as categorization or identification.

The next question that logically arises is "Why classify?" In general, there seem to be two basic reasons. The first is that the data base is too large to consider conveniently. Classification results in a decreased number of objects that, in turn, are more comprehensible and manageable. In this case, the classification process becomes an end in itself. A typical example might be the taxonomic classification of living things.

A second reason for classifying is that the subject matter is not well understood. It is hoped that the classification system will reflect the processes that have led to the observed arrangement, thus revealing a better understanding of natural laws. In this case, classification becomes a means to an end—a tool. It is the second reason that seems to have given impetus to many of the attempts at lake classification, particularly the geologically and hydrologically oriented systems.

Recently, considerations of lake management and protection strategies—especially those designed to abate cultural (accelerated) eutrophication—have provided additional, more pragmatic, incentives for devising lake-trophic classifications. This is particularly true in light of Section 314(a) of the Federal Water Pollution Control Act Amendments of 1972 (Public Law 92-500), which dictates: "Each State shall prepare or establish, and submit to the Administrator for his approval * * * an identification and classification according to eutrophic condition of all publicly owned freshwater lakes in such State * * *."

Such trophic-classification schemes are to be used to assess trends in lake quality and to aid administrators in environmental decisionmaking. Properly designed and employed, these schemes will allow for optimum management and protection of the lake resource.

CHARACTERISTICS OF CLASSIFICATION SCHEMES

Perhaps the most important distinction to make in regard to classification schemes is between monothetic and polythetic systems (Sokal, 1974). Monothetic systems require that classes differ from one another by a single property that is uniformly different among classes. Thus, lakes can be classified according to their stratification properties, as measured by the number of times the water column circulates annually. For example, the following scheme was proposed by Hutchinson (1957) and summarized by Wetzel (1975):

<i>Class designation</i>	<i>Description</i>
Amictic	Noncirculating; perennially frozen; Antarctic and some high-altitude lakes
Cold monomictic	One circulation period, in summer; temperatures never greater than 4°C; Arctic and high-altitude lakes
Dimictic	Two circulation periods, in spring and fall; temperate lakes and high-altitude lakes in subtropics
Warm monomictic	One circulation period, in winter; temperatures never less than 4°C; subtropical lakes
Oligomictic	Circulating rarely and at irregular intervals; temperatures well above 4°C; tropical lakes
Polymictic	Circulating frequently or continuously; lakes with large surface areas in windy regions of low humidity or high altitude; tropical lakes.

The above classes are subdivisions of either holomictic lakes, wherein the entire water column can circulate, or meromictic lakes, wherein a lower layer remains permanently stratified because of increased density from high dissolved-solids concentrations.

In polythetic classification systems, classes are defined on the basis of sharing a large proportion of similarities, but not necessarily having any property in common (Sokal, 1974). An excellent example of such a system is the classical scheme that has developed from the terms oligotrophic and eutrophic, as applied to lakewater types by Naumann (1919). Brief histories of the development of this scheme have been presented by Hutchinson (1969) and Rodhe (1969). Identification of lakes as eutrophic conjures up visions of (1) large concentrations or rates of input of nutrients, particularly phosphorus; (2) high rates of primary productivity, with associated large standing crops of algae (often blue-greens) and small Secchi-disc transparencies; (3) high rates of organic sedimentation; and (4) hypolimnia devoid of oxygen. It is

not necessary that a lake possess all these characteristics to be considered eutrophic, nor is it necessary that all eutrophic lakes possess any one of these characteristics. The nonuniformity of the trophic-classification scheme makes it particularly difficult to apply, although this is not its only disadvantage, as is explained below.

Uttormark and Wall (1975) discussed several characteristics of classification systems that also are worth summarizing. In post-set systems, classes are defined based on an analysis of the data base. Such systems are termed relative—lakes are classified with respect to one another, and the structure of the system may change when new lakes are added. This introduces an element of complexity when trying to apply such systems to data different from that used to derive the classification.

Preset systems, on the other hand, are composed of the predefined classes that do not depend on the data base. These systems are simpler to use than postset systems; a new lake can be added and categorized without changing the structure of the classification.

Uttormark and Wall (1975) described analytic systems—classes defined by a statistical or other numerical analysis of the data base—and empirical systems, whose class boundaries were defined arbitrarily.

In general, preset systems are empirical, and postset systems are analytic.

Finally, one should be aware of the differences between single-purpose and multi-purpose classification systems. Multi-purpose systems are more difficult to develop and generally become quite generalized. Such systems sacrifice utility for applicability to a broad spectrum of situations. On the other hand, the usefulness of lake classification often can be enhanced, if it is allowed to become dynamic, whereby single-purpose schemes are developed for specific applications. One must beware, however, of expending great amounts of resources trying to design an ad hoc scheme for each new situation. In reality, a compromise approach probably is best.

DESCRIPTIONS OF SOME LAKE-CLASSIFICATION SCHEMES

Most of the lake-classification schemes that have been developed probably can be described best as geologic, hydrologic, or trophic. Examples of each are summarized below.

GEOLOGIC SCHEMES

One of the most widely accepted lake classifications is that of lake-basin origin proposed by Hutchinson (1957). By considering the geologic processes responsible for lake-basin origin, the classification has been somewhat regionalized, as certain processes act predominantly on certain areas of the earth's surface. Hutchinson (1957) described 76 major basin types formed by tectonic activity, volcanic activity, landslides, glacial processes, rock solution, stream processes, wind, shoreline processes, organic accumulation, activities of higher organisms (including man), and meteoric impact. Twenty of the major basin types are of glacial origin, and Hutchinson (1957) noted that, "No lake-producing agent can compare in importance with the effects of the Pleistocene glaciations."

A second geologically oriented scheme, that may be useful in some areas, is based on altitude (Pennak, 1966). Four limnological zones were recognized in northern Colorado: (1) the plains zone, below 1,700 m (5,600 ft); (2) the foothills zone, 1,700–2,500 m (5,600–8,200 ft); (3) the montane zone, 2,500–3,200 m (8,200–10,500 ft); and (4) the alpine zone, above 3,200 m (10,500 ft). Generally speaking, lakes at higher altitudes are colder, less productive, and contain lower concentrations of dissolved solids than lakes at lower altitudes.

HYDROLOGIC SCHEMES

Born and others (1974) devised a hydrologic classification based on 63 glacial lakes located mostly in the north-central United States and south-central Canada. The incentive for the classification was based on the premise that certain hydrologic data are required to adequately define the nature and causes of lake-quality problems, and, thus, to outline appropriate steps for lake management and protection.

Lakes were classified on the basis of three descriptors: (1) regime dominance, that is, whether a lake's water budget is surface-water dominated (drainage lakes) or ground-water dominated (seepage lakes); (2) system efficiency, or flushing rate of water through the lake; and (3) position of the lake in the regional ground-water flow system. The latter determination is important for seepage lakes; it describes whether ground-water flows primarily from the aquifer to the lake (discharge lake), from the lake to the aquifer (recharge lake), or into the lake on one side and from the lake on the opposite side (flow-through lake).

In developing their classification, Born and others (1974) allowed for input of data or, because actual measurements often were not available, for input of descriptive information. The latter is basically an inference or best guess as to the functioning of the hydrologic system based on proxy criteria established by Born and others (1974). The available hydrologic data base was judged too sparse to adequately assess the performance of the proxy criteria, and it was concluded that " * * * a useful and workable classification cannot be adopted at this time." System efficiency seemed to be the most difficult of the descriptors to evaluate on the basis of the proxy criteria.

In Wisconsin, extreme lake levels leave boat docks high and dry in some years and cause flooded shorelines in others. In order to arrive at a better understanding of these stage fluctuations, Novitzki and Devaul (1978) devised a hydrologic classification similar to, but simpler than, that of Born and others (1974). Lakes were designated as surface-water flow-through (major surface inlet and outlet), ground-water discharge (surface outlet only), and ground-water flow-through or landlocked (no surface inlet or outlet). Landlocked lakes have the largest water-level fluctuations, and ground-water discharge lakes have the smallest fluctuations. The implications of using such a scheme, based on simple observations, for management purposes are obvious and illustrate the practical utility of lake classification.

Both of the above schemes employ a somewhat arbitrary determination as to which hydrologic variables should be used to classify lakes: the classifications are preset. Winter (1977) used a more general, postset, analytic approach to determine which variables are important. His study of 150 randomly selected natural lakes in north-central United States assumed that there existed no established variables upon which to base a hydrological classification. Winter (1977) selected all the variables " * * * believed to control the atmospheric, surface-, and ground-water interchange with a lake." His list of 13 variables included 1 relating to atmospheric-water interchange, 3 relating to surface-water interchange, 6 relating to ground-water interchange, and 3 relating directly to water quality and indirectly to ground-water interchange. Thus, the input data were weighted heavily toward the ground-water component of the lake's hydrologic budget.

Numeric data were available for some of the variables, the rest were subdivided based on qualitative descriptions, and numeric values were

assigned to each subgroup. The entire data set was subjected to principal-component analysis—a multivariate statistical technique (see, for example, Rummel, 1970) designed to disclose the important independent variables controlling the variance in a large complex data matrix. It was concluded (Winter, 1977) that precipitation-evaporation balance, ground-water dissolved-solids concentration, stream outflow, relation of drainage-basin area to lake area, position (altitude) in the regional ground-water flow system, regional slope, and local relief of the lake's drainage basin all should be considered in a general hydrologic classification of lakes. Stream inflow and drift texture (a measure of hydraulic conductivity) might be considered important, depending on how the results of the principal-component analysis are interpreted. Thus, the number of variables required was reduced from 13 to 7 or 9.

TROPHIC SCHEMES

Before discussing trophic classification schemes, it would be appropriate to examine briefly the lake trophic-state concept, as traditionally perceived, and some of its implications.

First, a useful distinction to be made is that between eutrophication and dystrophication. Reviews of the details of these processes have been presented by Rodhe (1969) and Wetzel (1975). It is important to note that the two processes follow separate pathways originating from a similar oligotrophic beginning (fig. 2); one process is not an extension of the other. When the rate of supply of organic matter to a lake comes predominantly from the watershed (allotrophy), the resulting process is dystrophication. When the rate of organic-matter supply results primarily from primary productivity of phytoplankton in the lake (autotrophy), eutrophication occurs.

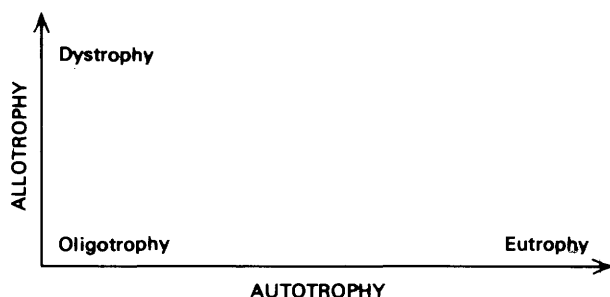


FIGURE 2.—Summary of the major trophic types of lakes (modified from Rodhe, 1969, and Wetzel, 1975).

A second important point is that both eutrophication and dystrophication are continuums. Note, for example, the graphical representation of the eutrophication process offered by Hasler (1947) (fig. 3). The result of this continuum realization has been an expansion of the terminology of Naumann (1919) to include mesotrophy, for those lakes having characteristics of both oligotrophy and eutrophy, and, more recently, ultraoligotrophy, hypereutrophy (presumably describing the effects of cultural or accelerated eutrophication), and other variants.

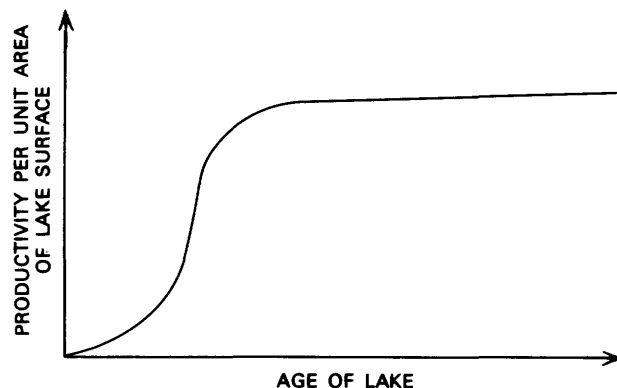


FIGURE 3.—Hypothetical curve of the course of natural eutrophication of lakes (modified from Hasler, 1947).

Finally, one must be aware of the controls that can be imposed on the eutrophication process by morphometry, particularly lake depth. Odum (1971) has presented the conception outlined in figure 4. Thus, for example, a lake can receive large quantities of nutrients; but if the lake is deep, the volume of water in the lake will keep the concentrations low enough so problems do not develop.

All the above points are reflected in current lake trophic classifications. Any such scheme ideally should accommodate both eutrophication and dystrophication; however, as the latter process is rarer, produces fewer esthetically displeasing results, and, most importantly, is not accelerated

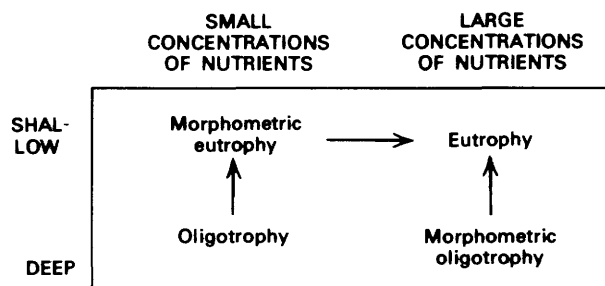


FIGURE 4.—Effect of lake depth on the process of eutrophication (modified from Odum, 1971).

by man's activities, it normally has not been considered. Also, most recent trophic classifications have taken a quantitative approach, in an attempt to avoid some of the subjectivity involved in determining when a lake changes from oligotrophic to eutrophic. Morphometric oligotrophy (fig. 4) creates particular problems when trying to apply some of the trophic-classification schemes.

Classifications of lake trophic-state might be based on physical, chemical, or biological measurements, or on various combinations of these. In one scheme requiring only chemical measurements, Zafar (1959) proposed a differentiation of trophic states using Pearsall's (1922) cation ratio, $([Na] + [K]) / ([Ca] + [Mg])$, where the concentrations are expressed on an equivalent basis:

<i>Trophic state</i>	<i>Pearsall's cation ratio</i>
Basically oligotrophic	> 2.0
Basically mesotrophic	1.2-2.0
Basically eutrophic	< 1.2

This classification depends on Pearsall's (1922) conclusion that waters with high ratios normally contain small concentrations of nitrogen, silica, carbonate, and phosphorus, whereas, waters having low ratios usually are rich in these nutrients.

Shannon and Brezonik (1972) used several multivariate statistical procedures to classify 55 lakes in north and central Florida. An expression for a trophic-state index (TSI) was derived using principal-component analysis:

$$TSI = 0.919 \frac{1}{SD} + 0.800COND + 0.896TON + 0.738TP + 0.942PP + 0.862CHA + 0.634 \frac{1}{CR} + 5.19,$$

where: SD = Secchi-disc transparency, in meters,
 COND = specific conductance, in micromhos per centimeter at 25°C,
 TON = total organic-nitrogen concentration, in milligrams per liter of nitrogen,
 TP = total phosphorus concentration, in milligrams per liter of phosphorus,
 PP = primary productivity, in milligrams per cubic meters per hour of carbon,
 CHA = chlorophyll *a* concentration, in milligrams per cubic meter, and
 CR = Pearsall's (1922) cation ratio.

TSI's ranged from 1.3 to 22.1; they were divided into five groups and labeled as follows:

<i>Trophic state</i>	<i>TSI</i>
Ultraoligotrophic	TSI < 2
Oligotrophic	3 > TSI ≥ 2
Mesotrophic	7 > TSI ≥ 3
Eutrophic	10 > TSI ≥ 7
Hypereutrophic	TSI ≥ 10

The classification of Shannon and Brezonik (1972) is a postset system: adding more lakes to the data base could change the classification structure. Thus, this system cannot be applied directly to other areas. One also should appreciate the fact that the same variables used by Shannon and Brezonik (1972) may not be appropriate trophic-state indicators in other regions; only the approach may be applicable.

Bortleson (1978) used a similar technique to classify lakes in the State of Washington. He calculated a trophic-state index, called a characteristic value (CV), based on principal-component analysis of 617 lakes:

$$CV = 59.4 \left(\frac{1}{SD} - 0.661 \right) + 74.9(UON - 0.697) + 126(UP - 0.124) + 104.1,$$

where: SD = Secchi-disc transparency, in meters,
 UON = total organic-nitrogen concentration in the upper waters, in milligrams per liter of nitrogen, and
 UP = total phosphorus concentration in the upper waters, in milligrams per liter of phosphorus.

Values of CV ranged from 1 to 1,259. Bortleson (1978) did not label CV ranges according to traditional trophic-state designations; however, lakes with larger values of CV normally would be considered more eutrophic. Bortleson's (1978) classification is a postset scheme.

A somewhat simpler approach to the trophic classification of lakes has been presented by the U.S. Environmental Protection Agency (1974) and was based on 209 lakes sampled by the National Eutrophication Survey staff in 1972. A trophic index number (TIN) was derived from annual median concentrations of total phosphorus (mg P/L), dissolved phosphorus (mg P/L), and inorganic nitrogen (mg N/L), 500 minus the annual mean Secchi-disc transparency (inches), 15 minus the minimum hypolimnetic dissolved-oxygen concen-

tration (mg/L), and the annual mean chlorophyll *a* concentration ($\mu\text{g/L}$). For each lake, each variable was ranked according to the percentage of lakes having a greater value. Thus, for example, a lake having a low total phosphorus concentration would have a high percentile rank for that variable. The TIN is the sum of the percentile ranks for each of the six variables; the more eutrophic lakes have lower TIN's.

Using a data set with 209 observations, the TIN can vary from 594 (six variables \times 99 percent) to 0. Actual values were in the range 573–41 and were labeled as follows:

<i>Trophic state</i>	<i>TIN</i>
Oligotrophic	> 500
Mesotrophic	499–420
Eutrophic	< 420

The U.S. Environmental Protection Agency (1974) emphasized: “ * * * the [trophic] index number ranges * * * are only valid when used with the data base of 209 lakes and reservoirs considered in this report. If a different data base were used, * * * the resulting index number might be interpreted quite differently * * *.” This classification is also a postset system.

An illustration of a preset system is provided by Bortleson and others (1974) in an early attempt at classifying Washington lakes according to trophic condition. Each of 24 separate variables was rated from 1 to 5, based on actual measurements, higher values indicating more eutrophic conditions. The variables were separated into: (1) natural physical factors, predominantly measures of lake morphometry; (2) culturally related factors, which represent nutrient-supply rates from various sources; and (3) trophic-state indicators, which are measures of chemical, physical, and biological conditions in the lake. A summed score was determined for each of the three groups of factors, and each score was interpreted separately.

Because of the number of variables involved, application of the system requires an exhaustive data-collection effort. Bortleson and others (1974) applied the scheme to seven Washington lakes to demonstrate the technique; however, they did not critically evaluate its effectiveness or its transferability to other regions.

The classification of 1,147 Wisconsin lakes was accomplished by Uttormark and Wall (1975) using an approach similar to that of Bortleson and others (1974). The variables considered (with their rating scales in parentheses) are: (a) hypolimnetic

dissolved-oxygen concentration (0–6); (b) Secchi-disc transparency (0–4); (c) history of fishkills (0–4); and (d) assessment of recreational use impairment by algal blooms or emergent vegetation (0–9). A larger rating implies a more eutrophic condition. The ratings from each of the four variables are summed to yield a lake-condition index (LCI).

The technique of Uttormark and Wall (1975) is much less data-intensive than that of Bortleson and others (1974). In addition, the LCI technique was designed to allow for substitution, when necessary, of subjective information for actual data. This characteristic permitted a considerably larger number of Wisconsin lakes to be classified than would have been possible otherwise.

According to Uttormark and Wall (1975) “ * * * LCI values are not synonymous with trophic status * * *,” however, the following comparisons were offered:

<i>Trophic state</i>	<i>LCI</i>
Very oligotrophic	0–1
Oligotrophic	2–4
Mesotrophic	5–9
Eutrophic	10–12
Very eutrophic	> 13

LCI's for Wisconsin lakes ranged from 0 to 22; and, for 42 lakes classified both by Uttormark and Wall (1975) and by the U.S. Environmental Protection Agency (1974), the following results were noted:

<i>Trophic state</i>	<i>Number of Lakes</i>	
	<i>LCI method (Uttormark and Wall, 1975)</i>	<i>TIN method (U.S. Environ- mental Protection Agency, 1974)</i>
Oligotrophic -----	5	2
Mesotrophic -----	16	5
Eutrophic -----	21	35
Total -----	42	42

Thus, the LCI method seems to be more lenient for assessing trophic state than the TIN method.

Uttormark and Wall (1975) also evaluated the possibility of using the LCI technique in other parts of the United States. They determined that the method would have direct applicability in the northeast and northern midwest, and would least likely be useable in the south and southwest. They indicated that the method might be useable, with some modification, throughout the rest of the country.

Thus far, one pervading point must be noticed: Except for Zarfar's (1959) scheme based on Pearsall's (1922) cation ratio, Secchi-disc transparency has been incorporated into all the trophic classifications discussed. Therefore, it is not surprising that a system has been developed (Carlson, 1977) based entirely on this measurement. The use of a single variable upon which to base a trophic-state index greatly simplifies data collection, and leads to somewhat less ambiguity than indexes based on several variables. The choice of Secchi-disc transparency is logical for a single-variable system, as Carlson (1977) notes that transparency has been related to hypolimnetic oxygen deficit (Lasenby, 1975) and to chlorophyll *a* and total phosphorus concentrations. All these latter variables have been incorporated into one or more of the previously discussed indexes.

Carlson's (1977) trophic-state index is computed from the equation $TSI(SD) = 10(6 - \log_2 SD)$, (3) where: SD = Secchi-disc transparency, in meters. By taking the logarithm to the base 2 of the Secchi-disc transparency, each doubling or halving of the transparency, which is assumed to reflect a corresponding doubling or halving of the algal biomass, causes the TSI(SD) to change by one unit. Subtracting from 6 ($= \log_2 64$) assures that the index is always positive (a transparency of 64 meters should never be exceeded) and inversely related to transparency. Multiplying by 10 allows one to carry 2 significant digits without including a decimal point. The index ranges from 0, at a Secchi-disc transparency of 64 meters, to a practical limit of about 100 (at a transparency of 62 mm):

<i>TSI(SD)</i>	<i>Secchi-disc transparency, in meters</i>
0	64
10	32
20	16
30	8
—	—
—	—
—	—
100	0.062

Carlson's (1977) index also can be calculated from chlorophyll *a* or total phosphorus concentration, based on generalized regression equations relating each of these variables to Secchi-disc transparency; indexes calculated in this manner normally are labeled TSI (Chl) and TSI (TP), respectively.

An advantage of computing the trophic-state index from Secchi-disc transparency is that the re-

quired data are simply and cheaply collected. The total phosphorus index is most stable seasonally; however, this advantage must be weighed against the probability that the general regression equation presented by Carlson (1977) actually describes the Secchi-disc total phosphorus relationship for the particular lake in question. Alternatively, the actual relationship for any given lake could be defined, but this makes the classification effort more complex and expensive.

Although Carlson (1977) did not label ranges of his index in terms of traditional trophic-state terminology, these relationships can be inferred as follows. Vollenweider (1968) suggested a classification of European lakes into ultraoligotrophic, oligomesotrophic, mesoeutrophic, eupolytrophic, and polytrophic based on total phosphorus concentrations. His mesoeutrophic class is defined by total phosphorus concentrations of 10 to 30 mg P/m³ and corresponds to the mesotrophic label of Sakamoto (1966) for Japanese lakes having roughly the same range of total phosphorus concentrations. By converting the total phosphorus concentrations to values of TSI(TP) (Carlson, 1977), one arrives at the following tentative boundaries for trophic states:

<i>Trophic state</i>	<i>TSI(TP)</i>
Oligotrophic	< 37
Mesotrophic	37–53
Eutrophic	> 53

In his review of lake-classification schemes, Shapiro (1975) noted that Carlson's index is " * * * applicable to many lakes." Indeed, it may be the most widely used system currently employed. Applications in Maine (Bailey, 1975), the Great Lakes states (Garn and Parrott, 1979), and Colorado (L. J. Britton and D. A. Wentz, written commun., 1980) are known. Many others undoubtedly exist.

Trophic classifications based primarily on biological data have been much less rigorous and quantitative than those previously discussed. The use of algal indicators has been discussed by Rawson (1956), who summarized the following major differences between trophic states:

<i>Algal measurement</i>	<i>Trophic state</i>	
	<i>Oligotrophic</i>	<i>Eutrophic</i>
Quantity -----	Poor	Rich
Variety -----	Many species	Few species
Waterblooms -----	Very rare	Frequent

Rawson (1956) noted that the few algal species often thought to be associated with eutrophic conditions may be a misnomer resulting from rarer species being overlooked in the relatively rich populations. He pointed out the importance of obtaining algal observations throughout the growing season, and provided a list of 18 algal indicators, arranged in order from oligotrophy through eutrophy based on dominant species in lakes of western Canada. Differentiation of trophic-state based on these indicators is not straightforward and should be approached with caution.

Tarapchak (1973) classified 68 Minnesota lakes as oligotrophic, mesotrophic-eutrophic, saline-eutrophic, and hypereutrophic on the basis of major solutes and the assumption that " * * * it is likely that salinity is in fact a good indirect measure of nutrient level." He investigated associated algal communities and found that the number of phytoplankton species was the only biological index sufficient for distinguishing the lake groups: diversity, evenness, standing crop, and various ratios (quotients) of numbers of individuals in certain taxonomic groups did not give satisfactory results. Of the 13 algal species Tarapchak (1973) found to be indicative of oligotrophy and the 9 species listed as representing eutrophy, only 1 species also was listed by Rawson (1956).

Primary productivity is a likely algal-associated variable for classifying lake trophic condition, especially given the concept in figure 2. Wetzel (1975) provided the following summary, based approximately on net primary productivity:

<i>Trophic state</i>	<i>Mean "net" primary productivity (mgC/m²/day)</i>
Ultraoligotrophic	< 50
Oligotrophic	50-300
Mesotrophic	250-1,000
Eutrophic	> 1,000

The greatest objection to this approach is the implicit assumption that organic matter inputs from littoral macrophytes and from sources in the lake's watershed are small, relative to those from the phytoplankton.

Relatively little has been published regarding trophic characterization of lakes on the basis of zooplankton. Ghilarov (1972) was successful in separating seven oligotrophic, morphometrically oligotrophic, mesotrophic, and morphometrically eutrophic lakes north of the Arctic Circle in northwestern Russia, using a plot of zooplankton biomass versus diversity. In addition, Brooks

(1969) summarized evidence indicating changes, due to complex competition-predation interrelationships, in copepod populations from the larger *Bosmina coregoni longispina* to the smaller *B. longirostris* during lake eutrophication. Thus, differences in zooplankton populations among lake trophic-states have been demonstrated; however, interpretation of lake trophic-state based on properties of zooplankton populations is not a well-defined procedure.

Shapiro (1975) reviewed several trophic-classification systems based on benthic macroinvertebrates and on fish production. He noted that these often are difficult to understand and apply, and that are not likely to have widespread application.

Finally, mention should be made of recent attempts to classify lake trophic condition from data collected by Landsat satellites. Multispectral scanners aboard Landsat measure intensities of light reflected from the earth in four wavelength bands: 500-600, 600-700, 700-800, and 800-1,100 nanometers. Boland (1976) developed a trophic-state index based on principal-component analysis of ground truth data from 100 Minnesota, Wisconsin, Michigan, and New York lakes sampled by the National Eutrophication Survey staff in 1972. He then used multiple regression analysis to predict his trophic-state index (dependent variable) from the intensities of light in the four Landsat wavelength bands (independent variables) reflected from within the lakes. In Wisconsin, 1,380 of 3,100 lakes with surface areas greater than 8.1 hectares (20 acres) have been categorized according to trophic status using a similar approach (Holmquist, 1977; Holmquist and Scarpace, 1977; Martin and Holmquist, 1979).

Both the above studies found that Secchi-disc transparencies could be predicted from Landsat data. This would indicate that Carlson's (1977) trophic-state index also could be used in conjunction with Landsat data to classify lakes.

CONCLUSIONS

It is interesting to note that the writing of this paper has become, itself, an exercise in classification. Hopefully, the above classification and categorization of lake-classification schemes have provided enough illumination to penetrate much of the murkiness, but not so much as to make the subject totally transparent. Looking back on what has been said, perhaps the most obvious conclusion is that a large variety of lake-classification schemes have been proposed. Some have rather limited ap-

plicability; others appear to be adaptable to different situations. When the need for a lake-classification system arises, the most important point to remember is not to apply these schemes indiscriminantly. As in all scientific endeavors, the results obtained through lake classification are porportional only to the thought directed initially to the problem.

REFERENCES CITED

- Bailey, John, 1975, Proposed trophic classification of the great ponds of Maine: Augusta, Department of Environmental Protection Draft Report, 25 p.
- Boland, D. H. P., 1976, Trophic classification of lakes using Landsat-1 (ERTS-1) multispectral scanner data: U.S. Environmental Protection Agency Report EPA-600/3-76-037, 245 p.
- Born, S. M., Smith, S. A., and Stephenson, D. A., 1974, The hydrogeologic regime of glacial-terrain lakes, with management and planning applications: Madison, University of Wisconsin-Extension, Inland Lake Renewal and Management Demonstration Project Report, 73 p.
- Bortleson, G. C., 1978, Preliminary water-quality characterization of lakes in Washington: U.S. Geological Survey Water-Resources Investigations 77-94, 31 p.
- Bortleson, G. C., Dion, N. P., and McConnell, J. B., 1974, A method for the relative classification of lakes in the State of Washington from reconnaissance data: U.S. Geological Survey Water-Resources Investigations 37-74, 35 p.
- Brooks, J. L., 1969, Eutrophication and changes in the composition of the zooplankton, *in* Eutrophication—Causes, consequences, correctives: Washington, D.C., National Academy of Sciences, p. 236-255.
- Carlson, R.E., 1977, A trophic state index for lakes: *Limnology and Oceanography*, v. 22, p. 361-369.
- Garn, H. S., and Parrott, H. A., 1979, Recommended methods for classifying lake condition, determining lake sensitivity, and predicting lake impacts: Milwaukee, U.S. Forest Service, Hydrology Paper 2, 48 p.
- Ghilarov, A. M., 1972, A classification of northern lakes based on zooplankton: *Hydrobiological Journal*, v. 8, p. 1-8.
- Hasler, A. D., 1947, Eutrophication of lakes by domestic drainage: *Ecology*, v. 28, p. 383-395.
- Holmquist, K. W., 1977, The Landsat lake eutrophication study: Madison, University of Wisconsin, unpublished M.S. thesis, 94 p.
- Holmquist, K. W., and Scarpace, F. L., 1977, The use of satellite imagery for lake classification in Wisconsin: Madison, University of Wisconsin, Water Resources Center, Technical Report, WIS WRC 77-08, 35 p.
- Hutchinson, G. E., 1957, A treatise on limnology, Vol. I. Geography, physics, chemistry: New York, John Wiley & Sons, 1,015 p.
- 1969, Eutrophication, past and present, in Eutrophication—Causes, consequences, correctives: Washington, D.C., National Academy of Sciences, p. 17-26.
- Lasenby, D.C., 1975, Development of oxygen deficits in 14 southern Ontario lakes: *Limnology and Oceanography*, v. 20, p. 933-999.
- Martin, R. H., and Holmquist, K. W., 1979, Remote sensing as a mechanism for classification of Wisconsin lakes by trophic condition: Madison, Wisconsin Department of Natural Resources, 54 p.
- Naumann, Einar, 1919, Nagra synpunkter angående limnoplanktons okologi med sarskild hansyn till fytoplankton: *Svensk Botanisk Tidskrift*, v. 13, p. 129-163. (Some aspects of the ecology of the limnoplankton, with special reference to the phytoplankton, English translation by I. B. Talling, *Freshwater Biological Association*, no. 49.)
- Novitzki, R. P., and Devaul, R. W., 1978, Wisconsin lake levels—Their ups and downs: Madison, University of Wisconsin-Extension, Geological and Natural History Survey, 11 p.
- Odum, E. P., 1971, *Fundamentals of ecology*, 3rd edition: Philadelphia, W. B. Saunders Co., 574 p.
- Pearsall, W. H., 1922, A suggestion as to factors influencing the distribution of free-floating vegetation: *Journal of Ecology*, v. 9, p. 241-253.
- Pennak, R. W., 1966, Rocky mountain states, in Frey, D. G., ed., *Limnology in North America*: Madison, University of Wisconsin Press, p. 349-369.
- Rawson, D. S., 1956, Algal indicators of trophic lake types: *Limnology and Oceanography*, v. 1, p. 18-25.
- Rodhe, Wilhelm, 1969, Crystallization of eutrophication concepts in northern Europe, in *Eutrophication—Causes, consequences, correctives*: Washington, D.C., National Academy of Sciences, p. 50-64.
- Rummel, R. J., 1970, *Applied factor analysis*: Evanston, Ill., Northwestern University Press, 617 p.
- Sakamoto, Mitsuru, 1966, Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth: *Archives Hydrobiologiae*, v. 62, p. 1-28.
- Shannon, E. E., and Brezonik, P. L., 1972, Eutrophication analysis—A multivariate approach: *Journal of the Sanitary Engineering Division, American Society of Civil Engineers*, v. 98, Proceedings Paper 8735, p. 37-57.
- Shapiro, Joseph, 1975, The current status of lake trophic indices—A review: Minneapolis, University of Minnesota, Limnological Research Center, Interim Report No. 15, 39 p.
- Sokal, R. R., 1974, Classification—Purposes, principles, progress, prospects: *Science*, v. 185, p. 1,115-1,123.
- Tarapchak, S. J., 1973, Studies of phytoplankton distribution and indicators of trophic state in Minnesota lakes: Minneapolis, University of Minnesota, unpublished Ph. D. dissertation, 204 p.
- U.S. Environmental Protection Agency, 1974, An approach to relative trophic index for classifying lakes and reservoirs—Working paper no. 24: U.S. Department of Commerce, National Technical Information Service PB-242 336, 44 p.
- Uttormark, P. D., and Wall, J. P., 1975, Lake classification—A trophic characterization of Wisconsin lakes: U.S. Environmental Protection Agency Report EPA-660/3-75-033, 165 p.
- Vollenweider, R. A., 1968, Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication: Paris, Organization for Economic Cooperation and Development, 220 p.
- Wetzel, R. G., 1975, *Limnology*: Philadelphia, W.B. Saunders Co., 743 p.
- Winter, T. C., 1977, Classification of the hydrologic settings of lakes in the north central United States: *Water Resources Research*, v. 13, p. 753-767.

Zafar, A. R., 1959, Taxonomy of lakes: *Hydrobiologia*, v. 13, p. 287-299.

SUPPLEMENTARY REFERENCES

- Anderson, George, 1975, Classification of Wisconsin lakes by trophic condition: Madison, Wisconsin Department of Natural Resources Report, 107 p.
- Bogoslovsky, B. B., 1966, On water budget lake classification, in *Hydrology of lakes and reservoirs: Symposium of Garda*, International Association of Scientific Hydrology, Publication No. 71, v. 2, p. 910-917.
- Bright, R. C., 1968, Surface-water chemistry of some Minnesota lakes, with preliminary notes on diatoms: Minneapolis, University of Minnesota, Limnological Research Center, Interim Report No. 3, 58 p.
- Dean, W. E., and Gorham, Eville, 1976, Major chemical and mineral components of profundal surface sediments in Minnesota lakes: *Limnology and Oceanography*, v. 21, p. 259-284.
- Donaldson, J. R., 1969, The classification of lakes: in *Middlebrooks, E. J., Maloney, T. E., Powers, C. F., and Kaack, L. M., eds., Proceedings of the eutrophication-biostimulation assessment workshop: Berkeley*, University of California, Sanitary Engineering Research Laboratory, U.S. Department of Commerce, National Technical Information Service, PB-215 604, p. 171-185.
- Findenegg, Ingo, 1964, Types of planktic primary production in the lakes of the Eastern Alps as found by the radioactive carbon method: *Verhandlungen Internationale Vereinigung Limnologie*, v. 15, p. 352-359.
- Jarnefelt, H., 1958, On the typology of the northern lakes: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 228-235.
- Larkin, P. A., and Northcote, T. G., 1958, Factors in lake typology in British Columbia, Canada: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 252-263.
- Likens, G. E., 1975, Primary production of inland aquatic ecosystems, in *Lieth, Helmut, and Wittaker, R. H., eds., Primary productivity of the biosphere*: New York, Springer-Verlag, p. 185-202.
- Margalef, Ramon, 1958, Trophic typology versus biotic typology, as exemplified in the regional limnology of Northern Spain: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 339-349.
- Milligan, J. H., Warnick, C. C., and others, 1973, Recreation water classification system and carrying capacity: Moscow, University of Idaho, Research Technical Completion Report, 89 p.
- Moyle, J. B., 1956, Relationships between the chemistry of Minnesota surface waters and wildlife management: *Journal of Wildlife Management*, v. 20, p. 303-320.
- Patalas, Kazimierz, 1971, Crustacean plankton communities in forty-five lakes in the Experimental Lakes Area, Northwestern Ontario: *Journal of the Fisheries Research Board of Canada*, v. 28, p. 231-244.
- Pennak, R. W., 1958, Regional lake typology in northern Colorado, U.S.A.: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 264-283.
- Reynoldson, T. B., 1958, Triclad and lake typology in northern Britain - Qualitative aspects: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 320-330.
- Round, F. E., 1958, Algal aspects of lake typology: *Verhandlungen Internationale Vereinigung Limnologie*, v. 13, p. 306-310.
- Ryder, R. A., Kerr, S. R., Loftus, K. H., and Regier, H. A., 1974, The morphoedaphic index, a fish yield estimator - Review and evaluation: *Journal of the Fisheries Research Board of Canada*, v. 31, p. 663-688.
- Schindler, D. W., and Holmgren, S. K., 1971, Primary production and phytoplankton in the Experimental Lakes Area, Northwestern Ontario, and other low carbonate waters, and a liquid scintillation method for determining ¹⁴C activity in photosynthesis: *Journal of the Fisheries Research Board of Canada*, v. 28, p. 189-201.
- Szesztay, K., 1974, Water balance and water level fluctuations of lakes: *Hydrological Sciences Bulletin*, v. 19, p. 73-84.
- Weiss, C. M., and Kuenzler, E. J., 1976, The trophic state of North Carolina lakes: Raleigh, University of North Carolina, Water Resources Research Institute Report No. 119, 224 p.
- Zumberge, J. H., 1952, The lakes of Minnesota - Their origin and classification: *Minnesota Geological Survey Bulletin* 35, 99 p.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1981 - 341-614/224

