A Coordinator's Guide to Volunteer Lake Monitoring Methods

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Contents

1.
Introduction

Citizens by the thousands have joined in new and innovative lake monitoring programs of the Upper Midwest and many other areas of the country. Why?—because these people are concerned about the condition of their favorite lake or reservoir and want to take the time to learn and help. Statewide programs have been established in seventeen states, with more being developed. Most of these programs are administered by state agencies, while others are supervised by universities and nonprofit organizations.

It is particularly timely, therefore, to critically examine existing programs for areas of strengths as well as to identify potential areas needing improvement. The purpose of this report is to assist people who develop, manage and participate in volunteer lake monitoring programs by:

1. Presenting background information on several important lake water quality parameters that are monitored in volunteer programs;
2. Highlighting the monitoring methods undertaken by programs in the Upper Midwestern states of Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin;
3. Recommending specific methods that will enhance the reliability and usefulness of volunteer monitoring data; and
4. Discussing the use of trophic state analysis to aid in data interpretation.

This report is divided into seven chapters.

Chapter 1: Introduction

This chapter discusses the importance of goal-setting as the first step in developing a successful citizen volunteer lake monitoring program. The six Upper Midwest monitoring programs are introduced and the goals of each listed. The administrative setup and the parameters measured are also described.

Chapter 2: Secchi Disk

This chapter introduces the Secchi disk depth as the cornerstone instrument for most citizen volunteer lake monitoring programs. The theory behind the Secchi disk measurement is presented along with a discussion of factors that impact measurement results. Secchi disk methods used by the programs in the Upper Midwest are also reviewed. The chapter concludes with recommendations for Secchi depth measurements.

Chapter 3: Chlorophyll

Chlorophyll is typically used as an estimator of algal biomass in lakes. This chapter defines the related chlorophylls and discusses methods of sampling, filtration and preservation. Methods used by the programs in the Upper Midwest are presented. Recommendations for the chlorophyll measurement are discussed at the end of the chapter.

Chapter 4: Phosphorus

Phosphorus is most often identified as the plant nutrient that limits algal growth in lakes. Chapter 4 describes the forms of phosphorus and procedures for analysis. Methods of sample collection in the Upper Midwest programs are described. The chapter concludes with recommendations for phosphorus measurement.

Chapter 5: Temperature and Oxygen

Temperature and oxygen concentration are two basic limnological variables that are not typically measured in volunteer monitoring programs. They play a very important role in the eutrophication process and

also dictate the presence or absence of various forms of aquatic life. These parameters are reviewed and alternative measurement methods discussed. Methods practiced in the Upper Midwest are presented along with recommendations for improving protocol.

Chapter 6: Sampling Strategies

This chapter outlines alternative strategies for determining when, where, and how to collect a water sample. Strategies of the Upper Midwestern programs are reviewed. The chapter concludes with several recommendations for sampling.

Chapter 7: Trophic State

Trophic state classification systems are popular tools for describing lakes. However, indices vary considerably in their approach and use of variables. This chapter gives perspective and guidance about various classification schemes. Trophic state classification recommendations for volunteer programs are highlighted.

Goal-Setting

Goals determine the character of individual citizen lake monitoring programs. They establish expectations, define monitoring parameters, and guide the development of sampling strategies. Goal-setting is a dynamic process. Goals often change as program and data needs change. Goals can also change as biases accompanying monitoring methods are eliminated, minimized or managed.

Vermont's lake monitoring program presents an example of how program feedback affects the nature of a program. The initial goals were to:

- Provide a regional definition of lake water quality;
- Define baseline water quality for individual lakes;
- Develop regional empirical eutrophication models; and
- Define lake water quality degradation/improvement detection tools.

Researchers emphasized that long-term monitoring is necessary to quantify lake variabilities (e.g., seasonal and year-to-year changes). Secchi disk transparency was identified as a major tool for individual lake monitoring efficiency and several years of data were typically recommended. Without several years of data collection, it may not be possible to statistically determine whether a lake has degraded or improved unless changes are extreme. **Given the reality of limited lake monitoring funds, volunteer efforts are critical to collecting quality scientific data on individual lakes.**

Programs in the Upper Midwest

This report reviews six statewide volunteer lake monitoring programs of the Upper Midwest to illustrate volunteer monitoring methodology. The programs included for discussion are:

- Illinois Volunteer Lake Monitoring Program (administered by the Illinois Environmental Protection Agency);
- Indiana Volunteer Lake Monitoring Program (administered by the School of Public and Environmental Affairs, Indiana University and the Indiana Department of Environmental Management);
- Michigan Inland Lake Self-Help Program (administered by the Michigan Lake and Stream Associations, Inc.);
- Minnesota Citizens Lake Monitoring Program (administered by the Minnesota Pollution Control Agency);
- Ohio Citizen Lake Improvement Program (administered by the Ohio Department of Natural Resources and the Ohio Lake Management Society); and
- Wisconsin Self-Help Lake Monitoring Program (administered by the Wisconsin Department of Natural Resources).

Throughout the remainder of this report the program names will be abbreviated. For example, the Michigan Inland Lake Self-Help Program will be identified simply as the "Michigan Program."

Three figures are presented to help the reader contrast and compare important features and historical milestones of the six statewide programs. Fig. 1.1 presents the parameters measured by volunteers in each program. Fig. 1.2 presents a timeline of the major parameters and important milestones of the programs. Fig. 1.3 summarizes the number of lakes included in each program along a timeline.

Illinois Volunteer Lake Monitoring Program

Goals

The Illinois Volunteer Lake Monitoring Program was created in 1981 by the Illinois Environmental Protection Agency (IEPA). The program operates with the following goals:

- To increase citizen knowledge and awareness of the factors that affect lake quality and usability so they can better understand the lake/watershed ecosystem and make informed decisions regarding lake use, protection and enhancement;

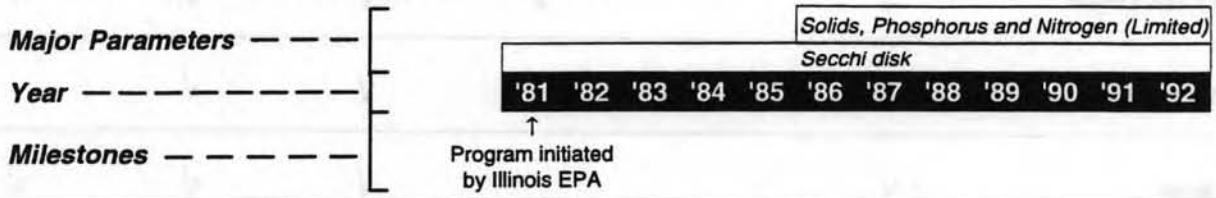
Table 1.1: Lake parameters measured by volunteer monitoring programs

	ILLINOIS	INDIANA	MICHIGAN	MINNESOTA	OHIO	WISCONSIN
Secchi Disk Depth	√	√	√	√	√	√
Station Depth	√			√	√	
Lake Level	√			√	√	√
Rainfall	√				√	√
Field Observations	√	√	√	√	√	√
Recreational Suitability				√		√
Water Color	√	√		√	√	√
Surface Temperature					√	(√)
Temperature Profile	(√)					(√)
Dissolved Oxygen Profile	(√)					(√)
Total & Volatile Suspended Solids	(√)					
Nitrogen	(√)					
Total Phosphorus	(√)	(√)	(√)			(√)
Chlorophyll *a*		(√)				(√)
Zebra Mussels	(√)					(√)
Loon Sightings				√ *(1987-1991)*		

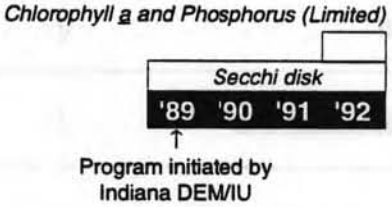
NOTE: (√) means that the parameter is measured only in the advanced sampling component of the program.

TABLE 1.2: Parameters and milestones of volunteer monitoring programs

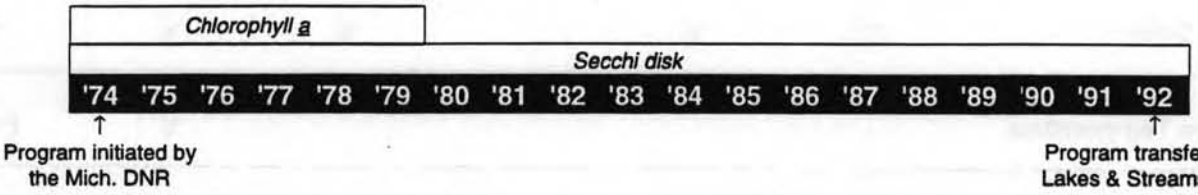
Illinois Volunteer Lake Monitoring Program



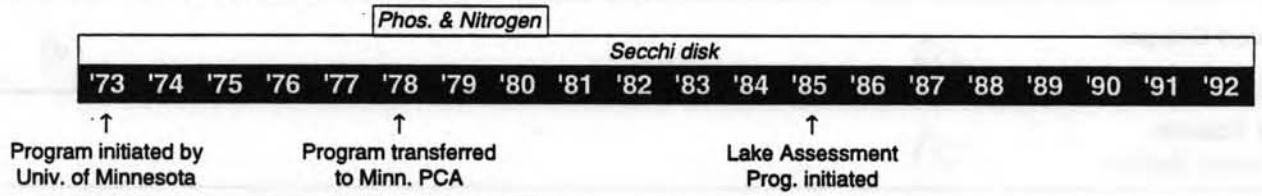
Indiana Volunteer Lake Monitoring Program



Michigan Inland Lake Self-Help Program



Minnesota Citizen Lake Monitoring Program



Ohio Citizen Lake Improvement Program

Wisconsin Self-Help Lake Monitoring Program

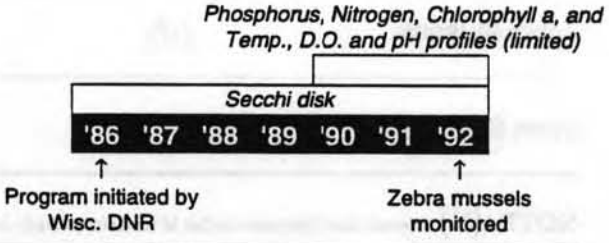
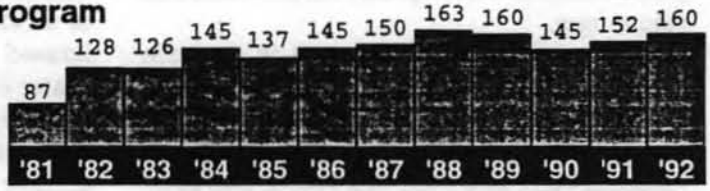
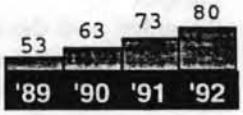


TABLE 1.3: Number of lakes monitored by volunteer monitoring programs

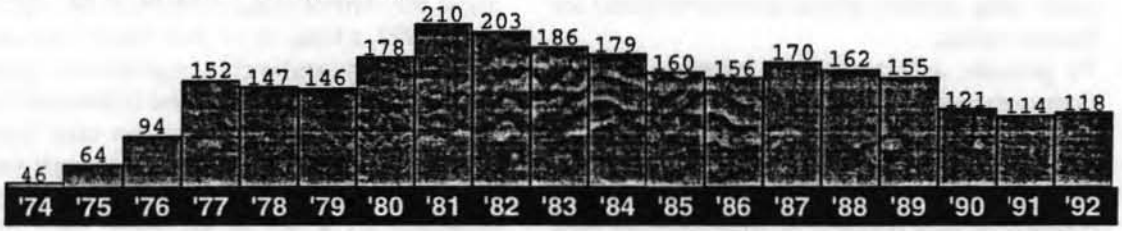
Illinois Volunteer Lake Monitoring Program



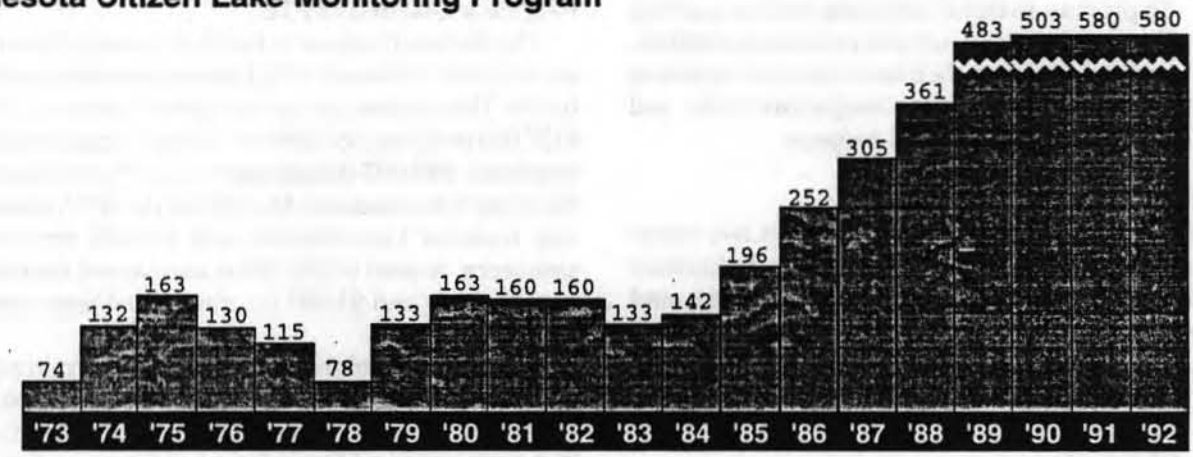
Indiana Volunteer Lake Monitoring Program



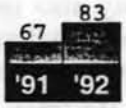
Michigan Inland Lake Self-Help Program



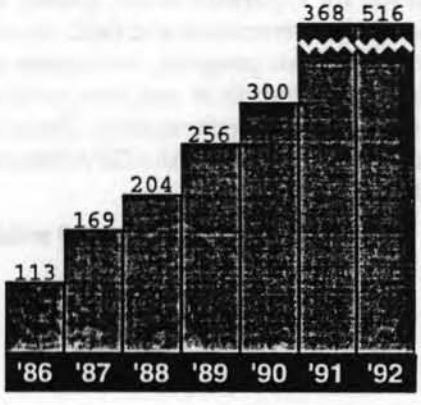
Minnesota Citizen Lake Monitoring Program



Ohio Citizen Lake Improvement Program



Wisconsin Self-Help Lake Monitoring Program



- To encourage development and implementation of sound lake protection and management plans to improve lake quality for intended and future uses;
- To encourage local involvement in problem solving by promoting local self-reliance and implementation through local resources, and to support these initiatives in lake protection /management and nonpoint source pollution control;
- To enlist and develop local "grass roots" involvement in environmental programs and foster cooperation among citizens, organizations and various units of government;
- To gather fundamental information (assessment data, routine physical observations) for Illinois' lakes;
- To provide a historic data baseline to help document water quality impacts, support lake management decision-making, target resources to solve identified problems and trigger further investigations, as well as to evaluate the effectiveness of lake protection/management procedures as implemented; and
- To provide an initial screening tool for guiding the implementation of lake protection/restoration techniques and a framework for a technical assistance program for cooperative lake and watershed management projects.

Parameters Measured

Illinois Program officials currently run two variations of the program. In the basic program, volunteers monitor their stations twice a month between May and October for the following parameters.

- Secchi disk transparency
- Station depth
- Lake level
- Water color

They also record field observations concerning weather conditions, the previous week's precipitation and qualitative assessments of aquatic plant populations. The basic program has operated continuously since 1981. A limited number of lakes (30 - 50 per year) are chosen for expanded water quality sampling. In addition to the parameters and field observations conducted in the basic program, volunteers collect water samples once a month at one, two, or three sites (depending on lake morphometry). These samples are subsequently analyzed in the IEPA laboratory for the following parameters.

- Total and volatile suspended solids
- Ammonia nitrogen
- Nitrate + nitrite nitrogen
- Total phosphorus

Administration

The Illinois Program is administered by staff members of the Illinois Lakes Program under the leadership of a Statewide Coordinator. The Lakes Program is housed in the Planning Section of the Division of Water Pollution Control, IEPA. Program staff are assisted by Areawide Planning Commission personnel (designated under Section 208 of the Clean Water Act) in three of the six regions of the state. IEPA contracts with these commissions to administer aspects of the program including training and follow-up visits. Each Commission is also responsible for preparing an annual regional program report. In the three regions that do not have an Areawide Planning Commission, personnel from the state Office of Community Relations assist the central office with training and reports.

In 1992, a total of 3.0 Full Time Equivalence (FTE) (i.e., person-years) were assigned to the program. This includes within the IEPA the Statewide Coordinator (0.7 FTE), three Lakes Program staff (totalling 0.75 FTE), three clerical and summer staff (totalling 0.5 FTE) and three Community Relations Coordinators (totalling 0.3 FTE). In addition, each of the three Areawide Planning Commissions are assigned 0.25 FTE, for a total of 0.75 FTE.

The Illinois Program is funded through Clean Water Act Section 106 and 205(j) grants and state matching funds. The current annual program budget of about $127,000 includes; $30,000 for Lakes Program staff and overhead, $45,000 distributed to the three Areawide Planning Commissions, $15,000 for the IEPA Community Relation Coordinators and $12,000 for clerical assistance. A total of $20,000 is earmarked for laboratory analysis and $1,000 for the annual lakes conference.

The targeted volunteers in the Illinois Program are citizens, especially those people who belong to lake associations. Public water supply operators, park district personnel and Department of Conservation employees are also encouraged to participate. Volunteers are recruited through the State agency newsletter and general press releases. The program currently operates at maximum capacity; therefore, recruitment is presently targeted only for lakes of special interest to IEPA. New volunteers are trained individually by program staff members at their lake. They receive all their equipment at no charge and pay no costs to participate in the program.

Over 334 lakes and six hundred volunteers have participated in the Illinois Volunteer Lake Monitoring Program since 1981. A total of one hundred sixty lakes were included in the program in 1992.

Indiana Volunteer Lake Monitoring Program

Goals

The Indiana Volunteer Lake Monitoring Program began in 1989 as a joint effort between the School of Public and Environmental Affairs (SPEA) at Indiana University and the Office of Water Management within the Indiana Department of Environmental Management (IDEM). It is a component of a comprehensive state Clean Lakes Program. IDEM contracts with SPEA to design and administer most aspects of the volunteer monitoring effort including volunteer training, data collection and management, and report writing.

In designing the program, IDEM officials drew on the experiences of several other volunteer monitoring programs. Assistance from the Vermont Lay Monitoring Program and the Wisconsin Self-Help Lake Monitoring was especially helpful. Officials strive to accomplish four major goals in the operation of the program:

- To collect water quality data that will contribute to the understanding of how Indiana lakes function;
- To monitor water quality changes to provide an early warning for problems that may be occurring in lakes;
- To encourage citizen involvement in the protection and management of their lakes; and
- To provide the means whereby Indiana citizens can learn more about how lakes function.

Parameters Measured

SPEA currently runs two versions of the volunteer monitoring program. In the basic program, volunteers measure the following parameters once every two weeks from May through October.

- Secchi disk depth
- Water color

In addition volunteers are requested to document other comments or observations made during the monitoring trip.

In 1992 the SPEA proposed, and the IDEM accepted to fund, a limited expansion of the program. Beginning in May, 1992, volunteers began collecting water samples monthly through October from stations on thirty lakes. These samples were analyzed in the SPEA laboratory for the following parameters.

- Chlorophyll *a*
- Total phosphorus

Administration

The Volunteer Lake Monitoring Program is administered by SPEA under the leadership of a Program Coordinator. The Coordinator spends approximately 0.1 FTE on the program. In 1992, six students of Indiana University worked part-time on the program, spending a total of approximately 1.0 FTE. The IDEM writes an annual contract to the SPEA to conduct the volunteer monitoring program. The funds for the program have come from the Clean Water Act Section 205(j) grants, Clean Lakes Lake Water Quality Assessment grants and state matching monies. The total annual budget for the program is approximately $20,000.

The targeted volunteers of the Indiana Program are citizens, especially lakeshore residents. Most of the lakes in the state are physically located in the northern one-third of the state. Consequently, there are significantly more lakes monitored in the northern counties than in southern counties. Volunteers are recruited through statewide news releases, announcements in the IDEM newsletter, Water Column; special mailings to the Water Column mailing list, special sessions and an informational booth at the annual Indiana Lake Management Conference, and by word-of-mouth.

New volunteers participating in the basic Secchi disk program are trained at the Indiana Lake Management Conference or in group training sessions held around the state by Clean Lakes Program staff. Volunteers participating in the expanded total phosphorus and chlorophyll *a* sampling program are trained individually by program personnel at their lake. Volunteers receive all the equipment at no charge and pay no costs to participate in the program.

In the first year of operation, forty-five volunteers monitored a total of fifty-three lakes. In 1992, sixty-five volunteers monitored eighty-six lakes.

Michigan Inland Lake Self-Help Program

Goals

The Michigan Inland Lake Self-Help Program is the second oldest volunteer monitoring program operating in the Upper Midwest. It was developed by the Inland Lakes Unit of the Michigan Department of Natural Resources (MDNR) in 1974 to gather background data on eutrophication trends. From 1974 to 1979, volunteers participating in the program took weekly water clarity measurements using a Secchi disk and collected a composite water sample from the euphotic zone (defined as twice the depth of the Secchi disk measurement). The samples were subsequently analyzed for chlorophyll *a* concentration.

The chlorophyll *a* portion of the program was eliminated at the end of the 1979 season because of increasing laboratory expenses and concern that there was not enough staff to properly conduct quality control activities. The Secchi disk portion of the program, however, was continued throughout the 1980s and into the 1990s.

In 1991 the nonprofit, citizen-based Michigan Lake and Stream Associations, Inc. (ML&SA) developed a free Secchi disk program for their membership. The

success of this program (coupled with the state's budget crisis) led to the transfer of the Self Help Program to ML&SA in 1992. Under the transfer agreement, the ML&SA accepts both members and nonmembers into the program.

The basic goals of the Michigan Program remain the same as they were established in 1972. That is:

- to provide long-term information to the state and lake users about water quality changes due to lake and watershed development; and
- to create an interest among residents and users about the quality of their lake resource.

Parameters Measured

Volunteers in the Michigan program monitor Secchi disk depth once a week from mid-May through early September. In addition, volunteers note any unusual conditions observed on the lake. In 1993, MDNR and ML&SA started an advanced Self-Help Program to measure total phosphorus at spring overturn. Participants in the advanced program are trained in sample collection and preservation protocol by MDNR scientists at ML&SA conferences. Samples collected by the volunteers are analyzed at the MDNR's state laboratory. ML&SA also established an optional water quality testing program for their member associations.

Administration

ML&SA is a membership organization open to all lake and/or stream associations, corporations and individuals. It was organized in 1961 as a nonprofit corporation for people and associations who desire to conserve and improve Michigan's lakes and streams and the Great Lakes and to protect and promote the wise use of water resources.

The administration of MDNR's Self-Help Program was transferred to ML&SA by Memorandum of Understanding. Even though the program is administered by a membership-based organization it remains an open program available to members and nonmembers. The MDNR continues to be involved in the program in areas of program planning and development and data review and evaluation. In the advanced program MDNR field offices are used as sample collection points and the laboratory provides analysis.

As a part of ML&SA's administration of the program they are responsible for overall program planning, information distribution, registration, data tabulation, report writing, and distribution of results. ML&SA charges an administration fee of $35 for the basic Secchi disk program and a fee of $15 for the advanced total phosphorus program. These are the only costs charged for the program. Other costs incurred by the volunteers are for purchase or construction of a Secchi disk and for transporting total phosphorus samples to MDNR's collection sites.

Michigan Program participants receive instructions on how to use the Secchi disk along with monthly report forms that are to be mailed back to program officials. Training also occurs at the annual fall meeting of the ML&SA, at the several spring regional meetings scheduled around the state and occasionally at member lakes. Volunteers are also encouraged to call ML&SA if they have any questions.

The MDNR estimates that over 520 lakes have been monitored at one time or another by program volunteers since 1974. A total of 118 lakes were included in the program in 1992, of those, only about twenty were not members of ML&SA.

Minnesota Citizen Lake-Monitoring Program

Goals

The Minnesota Citizen Lake-Monitoring Program (CLMP) is the oldest citizen volunteer monitoring program in the Upper Midwest. It was started in 1973 at the University of Minnesota Limnological Research Center (LRC) by Dr. Joseph Shapiro. A total of seventy-four lakes were monitored the first year. During 1978, because of the amount of response, the program became a joint effort of the LRC and the Minnesota Pollution Control Agency (MPCA). In 1979, the program was shifted to the Monitoring and Analysis Section of the Water Quality Division at the MPCA.

In a coincidence of timing, the state's ban on phosphorus in detergents had gone into effect the year the program was transferred. To learn more about the impacts of the ban, the MPCA expanded the program to include the collection of water samples on a select number of lakes. Samples collected in this advanced program were analyzed at the state Department of Health laboratory for total Kjeldahl nitrogen, total phosphorus, and color concentrations. Budget considerations and quality control problems were among the reasons that the advanced program was discontinued three years later.

Despite budget shortfalls and the lack of dedicated funding sources, the Secchi disk program was continued through the 1980s to 1992. For 1993, the Minnesota Lakes Association successfully obtained funding for a full-time coordinator form the Minnesota Legislature. The position also provides support for the Minnesota Lakes Electronic Bulletin Board (MN-Lake BBS) — a free service for those interested in Minnesota lakes.

The primary purpose of the program is to obtain basic lake water quality data on a large number of lakes that would otherwise go unmonitored if not for the participation of volunteers. The MPCA also recognizes that the program serves a larger role as an educational tool for helping individuals understand the connection between human activities in a lake's watershed and the resulting water quality.

Parameters Measured

Volunteers are urged to take weekly Secchi disk measurements during the summer season. Volunteers also make subjective judgements on the physical condition and recreational suitability of their lake based on the amount of algae present at their sampling site.

Administration

Currently the Minnesota Program is administered by coordinators in each of the MPCA's regional offices, who mix the program activities in with their other duties. Overall coordination is done by the new coordinator in the St. Paul office. The program is funded by the Minnesota Legislature.

Volunteers include lakeshore property owners, governmental units (e.g., cities, counties, and park managers), and others who use and enjoy Minnesota's lakes. The program recruits new volunteers through press releases, the annual lakes conference, the program's newsletter, a brochure and, most importantly, word-of-mouth. MPCA provides participants with a Secchi disk, data recording form, a lake map and instructions. There is a onetime fee of $10 to cover the costs of the Secchi disk kit. From that point on the disk is the property of the volunteer and no other fees are collected. Volunteers also receive a copy of the annual report that is generated from the data they collect.

Over 900 lakes (of the 15,237 in the state) have been monitored at one time or another during the life of the program. Over one hundred fifty of these have a sufficient database (at least ten years of data) from which a trend analysis can be performed.

The Minnesota Department of Natural Resources, Division of Waters administers a program that recruits volunteers to record lake levels at approximately 600 permanent and temporary stations. Titled "Lake Level Minnesota," the program has been in operation since 1970. The purpose is to create permanent, credible, lake level records that can be used for creating computer simulation and prediction models of lake fluctuations. This information is subsequently used to avoid lake level fluctuation problems associated with flooding, drought-related access, and aesthetics.

Ohio Citizen Lake Improvement Program

Goals

The Ohio Citizen Lake Improvement Program was initiated in 1991 as a cooperative program between the Ohio Lake Management Society (OLMS), the Ohio Environmental Protection Agency (OEPA) and the Ohio Department of Natural Resources (ODNR). Its roots, however, go back to the volunteer monitoring program developed by the Northeast Ohio Four County Regional Planning and Development Organization (NEFCO) in 1988.

Using NEFCO's experience as a base, OLMS developed a state-wide monitoring program. In 1990, a Memorandum of Understanding was signed with ODNR and a grant secured from the George Gund Foundation for a three year pilot project. Funds also came from a Ohio EPA Section 319 grant and the ODNR Division of Soil and Water Conservation.

Program organizers envisioned the program as a long-term project that will eventually become self-supporting. The stated project goals are as follows:

"OH-CLIP will educate citizen volunteers about prevention and sources of lake pollution and the use of Secchi disks for making measurements of water quality. A statewide database will be compiled from the information. The project will have several purposes including forming the basis for the eventual organization and formation of lake districts as political subdivisions in the State of Ohio."

Program officials operate the program with four major objectives:

- To promote citizen awareness of the role of nonpoint source pollution in harming lakes;
- To foster a grassroots initiative to control nonpoint source pollution to lakes;
- To foster formation of lake management organizations at the local level to control nonpoint source pollution and improve water quality; and
- To foster educational opportunities for citizens of all ages concerning the lake as a living, outdoor laboratory.

Parameters Measured

Volunteers are instructed to measure the following parameters during the first and third weeks of each month from May through October.

- Secchi disk transparency
- Station depth
- Water color
- Water temperature (near surface)

Volunteers also record field observations including recreational use, cloud cover, wind direction, air temperature and the amount of rainfall for the three days prior to sampling.

Administration

The Ohio Program is officially housed in the Division of Parks and Recreation in the ODNR. But, in fact, the program is truly a cooperative effort between the ODNR, the Ohio Lake Management Society, a volunteer CLIP Advisory Board, OEPA and the Soil & Water Conservation District Offices.

The day-to-day business activities of the program are accomplished by a lake management specialist employed by OLMS (1/2 FTE) and a ODNR Parks and

Recreation employee (1/4 FTE). A retired Ohio State University professor, a Division of Soil and Water Conservation employee and other professionals also devote significant hours advising the program.

The Ohio Program is funded through a three year grant from the George Gund Foundation, annual US EPA 314 Lake Assessment grants, an Ohio EPA Section 319 grant and the ODNR Division of Soil and Water Conservation. ODNR provides office space and in-kind services. Volunteers are trained at workshops conducted around the state in April. In 1991, a total of two hundred and thirty-three people attended thirteen workshops to learn about the program and participate in training. Over one hundred and ten people subsequently sent in data for sixty-seven lakes.

Wisconsin Self-Help Lake Monitoring Program

Goals

The Wisconsin Self-Help Lake Monitoring Program was initiated in 1986 by the Wisconsin Department of Natural Resources (WDNR) as a component of a new state Lake Management Program. A primary thrust is to give citizens an active role in lake management activities. In addition, the program enables the WDNR to collect basic data on lakes that would otherwise go unmonitored.

The Wisconsin Program was designed with six objectives in mind:

- To teach citizen volunteers some concepts of basic limnology, how lakes in general work and to increase their understanding of the water quality of their lake in particular;
- To teach citizens about basic lake sampling techniques, specifically how to use a Secchi disk carefully, regularly and according to set procedures;
- To document changes in water clarity over time on a centralized computing system;
- To compare water clarity data for all the lakes in the program on both a regional and statewide basis; and
- To collect data accurately over time in order to make sound lake management decisions.

Parameters Measured

The WDNR runs three variations of the Wisconsin Program. One is a basic water clarity measurement program using a Secchi disk. Volunteers also record water color, perceptions of water quality, weather, and on some lakes, lake level. Volunteers are requested to sample their lakes at least every other wee.

A limited number of lakes are enrolled in an advanced water quality sampling program called the Trophic State Index (TSI) Program. In addition to Secchi disk measurements and the basic program field observations, volunteers are trained to use a VanDorn water sampler to collect water samples at specific depths in the water column. These TSI samples are collected five times a year: spring turnover (defined as two weeks after ice-out), June, July, August and October. Four parameters are measured.

- Secchi depth
- Water temperature (water column profile)
- Total phosphorus (top and bottom of water column)
- Chlorophyll *a*

The third variation of the Wisconsin Program is called the Expanded Monitoring. Volunteers obtain Secchi disk measurements, record field observations and undertake scheduled TSI sampling. Additionally they measure the following two parameters.

- Dissolved oxygen (water column profile)
- Rainfall (optional)

Zebra mussel monitoring is also conducted on a limited number of lakes in conjunction with the University of Wisconsin Sea Grant's Mussel Watch Program. The participating lakes are selected based on their "mussel-vulnerability" (i.e., lakes near the Great Lakes or Mississippi River). Volunteers place mussel samplers in the lakes and also walk the shore looking for mussels on rocks, logs, piers, etc. If mussels are found they scrape off some samples, place them in alcohol, and send it to the WDNR for verification.

Administration

The Wisconsin Program is housed in the Lake Management Program within the WDNR. A team of three Lake Management Specialists in the Madison office handle most administrative and technical aspects of the program. Lake Management Specialists in the six WDNR district offices train volunteers, maintain equipment, and conduct public education and other activities.

The targeted volunteers for the Wisconsin Program are citizens, especially those who live near or recreate on lakes. Volunteers learn about the program through a widely distributed fact sheet, contact with WDNR district and area offices, newspaper, magazine and radio stories, and by word-of-mouth.

Volunteers are trained on their lakes by a WDNR Lake Management Specialist or in a group session at a convenient location. Experienced volunteers occasionally trained other volunteers. Training manuals and data sheets are provided to all volunteers. Volunteers receive all equipment and supplies needed to conduct sampling at no cost.

2.
Secchi Disk

The Secchi disk is the cornerstone instrument in most volunteer lake monitoring programs. It is inexpensive to buy or to construct, requires little instruction to use, and does not require any consumable or dangerous supplies. It provides information that is both useful and easily interpreted by volunteers. Secchi transparency data serves as a key variable in a number of predictive and diagnostic equations as well. It can also be used to detect lake water quality trends.

The Secchi disk is named after Pietro Angelo Secchi, a papal scientific adviser and head of the Roman Observatory (Preisendorfer 1986a,b; Peskova 1991) (Fig. 2.1). The disk itself was devised by Commander Cialdi, the commander of the papal fleet (Tyler 1968). Cialdi asked Secchi to experiment with this disk in the coastal waters of the Mediterranean.

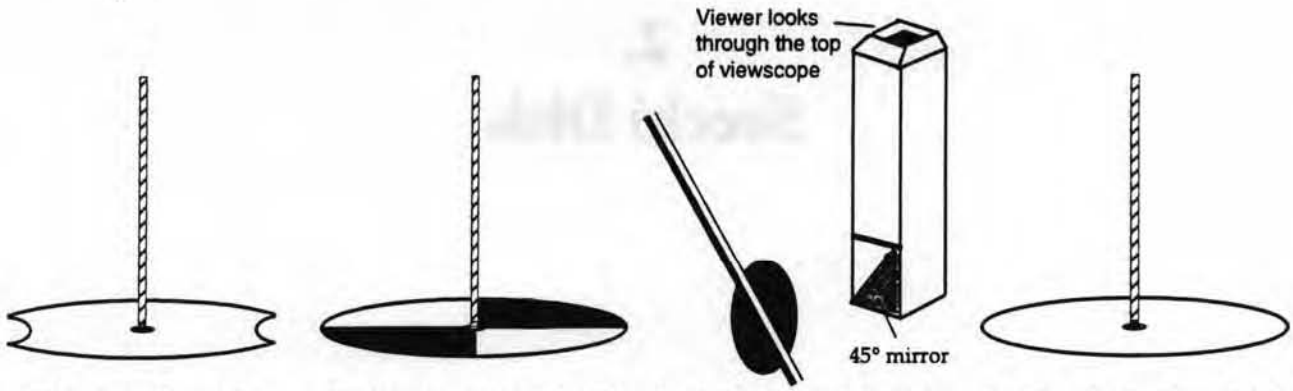
Figure 2.1.—Pietro Angelo Secchi

The first disk was lowered from the papal yacht, *l'Immacolata Concezione* on April 20, 1865. Actually, Secchi used two disks in his initial experiments. One was a 43 cm diameter white clay disk, the other was constructed of sail cloth, painted white, stretched on an iron ring 60 cm in diameter. According to Preisendorfer (1986a), Secchi's experiments included lowering the disks on the sunny and shady side of the ship, viewing the disappearance of the disk from different heights, shading the surface of the water with hats and umbrellas, and using yellow and brown-colored disks.

The disk has undergone few revisions since Secchi's time (Fig. 2.2). The standard size of the disk used in inland lake investigations has shrunk to 20 cm, but a variety of different sizes and constructions have been used. A 100 cm disk was used in Crater Lake (Larson 1972) and a 15 cm disk made from the plastic top of a margarine container was used in Lake Geneva (McCauley 1990). A large coffee can lid (12 cm) spray-painted white was used on the Great Lakes after someone forgot to bring along the standard disk (Schelske personal communication).

A variety of materials have been used to construct disks. Besides the margarine container top and the tin can lid mentioned above, Jones and Bachmann (1978) used white 19.5 cm plastic plates in their statewide study. Carlson once used a white Frisbee and Betz (Personal communication) reports a volunteer using a pizza pan painted black and white.

The commercial marine disk is still all-white, but a disk with alternating black and white quadrants has become the *de facto* standard in lakes. The black and



The Minnesota Citizens Lake-Monitoring Program uses an all-white 20 cm disk with notches on the side for line storage.

A 20 cm diameter disk with alternating black and white quadrants is typically used in freshwater lakes.

Davies-Colley (1988) describes the advantages of an all-black disk viewed through a scope.

An all-white 40 cm diameter disk is used primarily in marine environments.

Figure 2.2.—Variations of the Secchi disk

white disk was apparently devised by George Chandler Whipple, a professor of sanitary engineering at Harvard University. In his book, *The Microscopy of Drinking Water* (Whipple, 1899) Whipple recommends the use of a black and white disk in favor of a bright platinum wire, one millimeter in diameter, that was previously used by Alan Hazen. He states, "The author's experiments have shown that the limit of visibility may be determined most accurately by using a disk about 8 inches in diameter, divided into quadrants painted alternately black and white like a target of a level-rod, and looking vertically down upon it through a water-telescope provided with a suitable sunshade."

Shapiro *et al.* (1975) introduced the use of a notched all-white disk. The notches are used to wrap and store the cord. Davies-Colley (1988) devised an all-black disk, which is viewed horizontally by a diver or by using a horizontally oriented viewscope. The black disk is most suitable for rivers or other shallow environments, and is reported to provide better optical information than the Secchi disk.

Theory of the Secchi Disk

Despite the numerous advantages of the Secchi disk, the information it provides is often discounted as being of limited value. One reason for this is that the Secchi disk is one of the few remaining limnological instruments that has the observer as an integral part of the measurement process (Preisendorfer, 1986). Human visual acuity, differences in interpretation of the depth of disappearance, and even the nature of the visual mechanism can result in different Secchi disk readings by different observers viewing the same water body, even in the same boat. Besides potential human errors, factors such as the nature of the disk, glare on the water surface, time of day, and numerous other factors contribute to variability in transparency measurements. Curiously, there are few published accounts that describe the degree of severity of these problems.

This section presents the theory behind the operation of the disk. The point of this section is not to overwhelm the reader with equations, but to use the equations to highlight potential problems with the using the disk. We will only briefly review the factors that affect disk operation. Several excellent reviews of the theory behind the Secchi disk exist (Preisendorfer 1976, 1986a, and 1986b; Tyler 1968; Davies-Colley et al. 1993). The reader may refer to these for even more detailed accounts describing how a Secchi disk works.

The Secchi disk is a contrast instrument—the depth of disappearance of the disk is determined by the ability of the observer to discern the difference between the object (Secchi disk) and its ambient background. As illustrated by Williams (1970), this contrast relationship is represented as:

$$\text{Contrast} = \frac{\text{Object luminance} - \text{Background luminance}}{\text{Background luminance}}$$

This means that factors that affect the contrast or the observer's ability to view the disk will affect the apparent depth that the disk disappears. The theoretical basis of the disk is an equation by Duntley and Preisendorfer (1952) which describes the loss of contrast of a submerged object along a path with a vertical path of sight. In this equation:

$$C_R = C_O e^{-(\alpha+K)z} \quad (2.1)$$

C_R is the apparent contrast; C_O, the inherent contrast; z, the depth of disk disappearance (Secchi depth); α, the beam attenuation coefficient; and K, the vertical attenuation coefficient. Equation 2.1 can be rearranged to allow the examination of factors that affect the depth at which the Secchi disk disappears (Z_{SD}).

$$z_{SD} = \frac{\ln(C_O / C_R)}{(\alpha+K)} \quad (2.2)$$

Surface of the Disk

The inherent contrast, C_O, is the contrast of the Secchi disk as observed when positioned just below the water surface. This term incorporates the intrinsic properties of reflectance of the disk itself. Inherent contrast is affected by the degree of reflectance of the Secchi disk, which should be born in mind when assuming that any white paint can be used as the reflective surface of the disk. Disks should have a smooth, white matte surface, preferably of known and standardized reflectance.

Although little discussion can be found in the Secchi disk literature, the use of a matte rather than a glossy surface is important. A glossy surface will reflect light at an angle equal to that with which the light strikes the surface. Because of this, a substantial portion of the light will not be reflected upwards towards the observer unless the sun happens to be directly overhead (Fig. 2.3). The angle of the sun, and therefore the effect of time of day can cause more differences with a glossy disk than a matte disk. If you think about it, a mirror would look black except when the sun was directly above the observer. A perfectly diffusing surface, approximated by the matte finish, would appear equally bright regardless of the direction in which it is viewed (Wyszecki and Stiles 1982). A matte-surfaced disk should therefore be less sensitive to sun altitude than a glossy disk.

Time of Day

C_R is the apparent contrast of the disk as seen by the observer at the distance z (Tyler 1968). Preisendorfer (1986) replaced C_R with C_T, the *threshold contrast*, the value obtained when an observer, on repeated attempts under identical conditions to decide if the disk is seen, is correct 50% of the time. C_T (or C_R) is highly dependent on the visual acuity of the observer. The threshold contrast varies with the amount of ambient light and with the angle subtended by the target at the eye. At the illuminance levels usually found if the Secchi depth is measured at or near solar noon, the contrast threshold can be considered constant (Williams 1970). However, in the early morning and late afternoon the lessened amount of light reflecting directly upwards should result in lower Secchi readings.

Åberg and Rodhe (1942) found that readings taken in the late afternoon had smaller Secchi depths. Verschuur (1995) found that there is a marked difference in Secchi depths depending on the time of day (Fig. 2.4). The usual warnings about taking the reading between 10 AM and 3 PM should be emphasized to the volunteer. It might be worth considering either to correct early morning and late evening readings to solar noon values or to discard them. Vershuur (Personal communication) is attempting to produce correction equations.

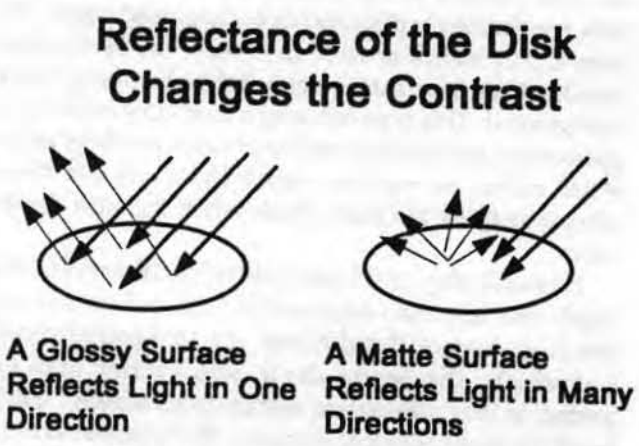


Figure 2.3.—A matte disk should equally distribute reflected light in all directions, making the disk reading less sensitive to sun angle.

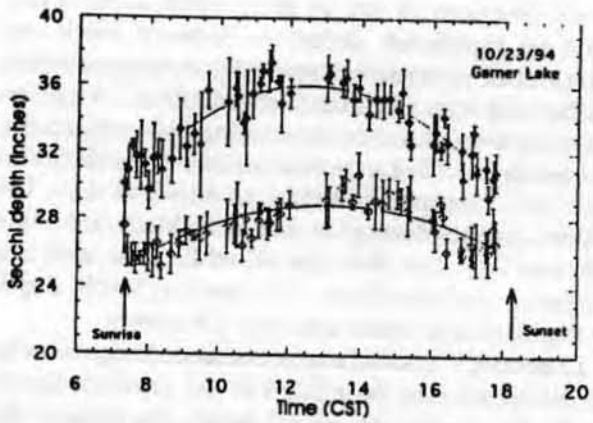


Figure 2.4.—Secchi depth measurements obtained October 23, 1994. The top curve shows data obtained with a viewing tube. The lower curve data were obtained without a viewing tube. Each point represents the average of eight measurements with the error bars indicating one standard deviation above and below the means. From Vershuur (1995).

Size of Disk

The angle subtended by the target at the eye means that the size of the disk as it disappears from site affects the reading. It would suggest that the larger the disk, the greater the depth at which it would be seen. Secchi, himself, found this to be the case. Larson (1972) found that a 20 cm disk gave a Secchi depth of 39 m in Crater Lake; a 100 cm disk increased the depth by 13%. Holmes (1970), however, found no difference in Secchi depth using 20, 30, and 51 cm disks in marine waters with Secchi depths of 5 meters. Preisendorfer (1986a) suggested that, although the theory predicts that disk size will affect Secchi depth, the effect is slight.

Davies-Colley (1991) recommends the use of a series of different sized disks, each used in a different range of Secchi depths, so that the apparent size of the disk when it disappears remains approximately constant. For example, the standard 20 cm disk should be used within the range of 1.5 to 5 meters (10 meter maximum). A 20 mm disk should be used from 0.15 to 0.5 m and a 60 mm disk, between 0.5 and 1.5 m. Although this recommendation may enhance the accuracy of the reading, it is most likely impractical for volunteer monitoring programs.

Color of the Disk

If the size of the disk is important, then the black and white disk, with its reduced amount of white reflective surface, might be expected to disappear sooner than an all-white disk. If this were true, then it is a strong argument for a standardized disk size and color. Jones and Bachmann (1978) however, found no significant difference in Secchi depths obtained with a slightly concave, off-white dinner plate and a black and white 20 cm Secchi disk over a range of 0 to 4 meters. Students of one of the present authors also found no significant difference between black and white and all-white disks in repeated measurements in an Ohio lake with a median Secchi depth of 1.9 meters. However, two volunteers monitoring Clearwater Lake, Minnesota provided data that indicate that a black and white disk disappeared before an all-white disk. The median depth of disappearance of the black and white disk was 7.6% less than the all-white disk, and this difference was significant. The median Secchi depth for the black and white disk was 9.9 meters.

LaBounty (Personal communication) suggests that the difference may be related to the absolute Secchi depth; the greater the actual depth, the greater the difference between the two disks. Davies-Colley (1991) recommends that a smaller disk be used as the transparency of the water becomes less. This is appropriate not only because of the need to keep the apparent size the same, but because the disk distorts the light field near the disk, and, at low transparencies, the glow above the disk increases the apparent visibility of the disk. Again, the need for simplicity and conformity may favor the use of a single-sized disk. The black and white disk is used extensively which suggests that it should become the recommended standard disk color.

Depth of Disappearance

Another factor affecting the C_T term is the time the observer takes looking for the disk. The longer the observer has to look for an object, the greater chance that he will see it (Williams, 1970). A correlated phenomenon is that Secchi depth should be greater if the observer continues to watch the disk as it descends into darkness than if he tries to find the disk as it reappears from the depths. Personal experience, contrary to theory, is that the depth of reappearance is usually greater than the depth of disappearance. Custer, Ohio-NEFCO, (Personal communication) suggests that this discrepancy might occur because it is easier to distinguish the lightening of the area above the disk against the black background on the ascent than to mark the point where the ever-darkening disk has equal luminance to the background. Betz (Personal communication), however, finds it easier to watch the disk disappear than to find it on the assent.

A number of programs measure, and then average, the depth of disappearance together with the depth at which it reappears. Other programs recommend measuring only the depth at which the disk disappears. Either method can be considered "correct," but the methods may yield different values.

Nature of the Background

Since the Secchi disk is a contrast instrument, the depth of disappearance will depend on the contrast of the disk relative to the background. As the contrast between the Secchi disk and its background varies, the term C_o/C_T varies as well. In theory, the Secchi disk should disappear into a total light absorbing black background. This type of background may exist in the open ocean, but in lakes and reservoirs, particles in the water reflect as well as absorb light and the background may be far from black when the disk disappears.

Davies-Colley and Vant (1988) found that the Secchi depth was less than expected in high reflectance waters (e.g., waters "brightened" by suspended clays). Conversely, the Secchi depth was greater than expected in low reflectance waters (e.g., waters "darkened" by highly colored humics). There seems to be no way to avoid this problem. The black sector of the black and white disk, however, might provide a more constant black background to the observer's eye.

Surface Reflectance

Preisendorfer (1986a, b) added another term (here designated as τ) to the Duntley equation (2.3) to account for the effects of "crinkling" of the water surface by waves and the reflection of skylight on a calm surface. With the addition of this term, the contrast equation becomes:

$$C_R = \tau C_o e^{-(a+K)z} \qquad (2.3)$$

And the Secchi disk equation becomes:

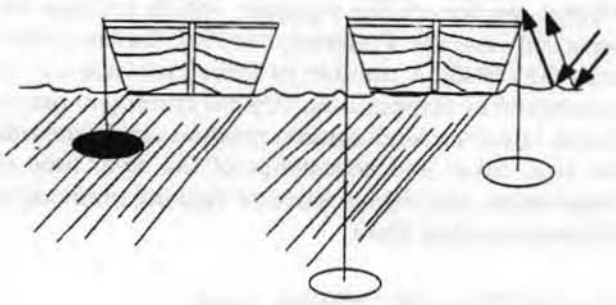
$$z_{SD} = \frac{\ln(\tau C_o / C_R)}{(a+K)} \qquad (2.4)$$

The potential impact of term, τ, invites discussion of whether the observer should wear sunglasses and if the observations should be taken on the sunlit or shaded side of the boat. If surface reflectance reduces the ability to see the disk, then it would seem that every effort should be made to minimize surface reflectance. This would suggest the use of polarized sunglasses to minimize glare. However, it might be that lowering the total luminance by using sunglasses will also reduce the ability of the observer to see the disk. The current standard practice is to remove sunglasses because they change the Secchi depth reading.

Lowering the disk on the shady side of the boat is often suggested to reduce surface glare. This tactic causes other problems, however. The shadow of the boat interferes with the light path and, depending on the Secchi depth, may shadow the disk itself (Fig. 2.5). Davies-Colley and Vant (1988) found that Secchi depths were similar or slightly shallower than expected if the observations were made on the shaded side and the disk itself was in the boat's shadow. If, however, the observations were made on the shaded side and the disk was clear of the boat's shadow, then the Secchi depth on the shaded side exceeded the sunny side depth.

In the first instance reported by Davies-Colley and Vant, the disk was shaded and therefore its contrast was lowered. In the second instance the sunlit disk was being viewed against a darker background of water in shadow. The darker background enhanced the contrast and therefore increased the Secchi depth. Davies-Colley and Vant estimated the error of viewing the disk on the shaded side of the boat but having the disk in the sun rather than in the boat shadow to be as much as 15 percent. To eliminate surface glare, yet keep the light path and disk clear of the boat's shadow, it might be best to view the disk on the sunny side of the boat, but use an underwater viewscope or a glass-bottomed bucket to eliminate all surface interference. However,

Figure 2.5.—The shadow of the boat can affect the Secchi depth. The direction of error will depend on whether or not the disk is in the shadow of the boat.



even the use of a viewscope appears to be controversial. Although several volunteer programs use them, Holmes (1970) found no significant differences in Secchi depth, whether or not a viewscope was used.

The Human Effect

The final interference factor to Secchi depth readings relates to the limitations of the human eye itself. The eye is not equally sensitive to all wavelengths of light. At high light levels, the maximum sensitivity is at 555 nm, in the yellow-green range (Williams 1970; Preisendorfer 1976). The effect of this varying sensitivity of the eye to different wavelengths is that the Secchi depth will vary depending on the color of the water through which the disk descends. This may not be a significant problem in lakes in which the most penetrating color is in the green wavelengths (Talling 1957). In humic-stained lakes where the color of the water is shifted even further away from the optimal visual sensitivity, however, the Secchi disk would disappear sooner than would be predicted based on the amount of dissolved and particulate matter in the water. Williams (1970) suggested that if the observer viewed the disk through a Wratten #61 optical filter, the filter would standardize the color that the observer would see and thus eliminate visual bias.

Summary

Before we can even begin to deal with the factors affecting transparency that are of interest to the volunteer and limnologist, the program coordinator must consider instrument and observer limitations. As mentioned above, these problems include the reflectance of

the disk, visual acuity of the observer, the conditions of observation, the amount of water surface reflectance, and the rippling of the surface layer. It may be that most of these problems cause only small errors in measurement as they are incorporated into the logarithmic portion of the equation which reduces the sensitivity to error (Preisendorfer 1986; Davies-Colley and Vant 1988). A number of these problems can be minimized by standardizing the measurement procedures, however. Such standardization would include the size, color, and reflectance of the disk, time of observation, and the adoption of viewing methods to minimize surface glare.

Secchi Depth, Algae, and Interfering Substances

The limnologist and volunteer are usually interested in using the Secchi depth to estimate the amount of algae in the water. This relationship is based on the idea that algal particles affect the penetration of light into the water and therefore, the Secchi depth. The penetration of light in water is affected by the magnitude of the terms α and K. The term α (*beam attenuation coefficient*), in the Duntley equation (2.2) represents the attenuation and scattering of light through a thin layer of water perpendicular to the light path, and the term K (*vertical attenuation coefficient*) represents the average attenuation and scattering of light through a vertical light path from the surface to the Secchi depth.

In essence, both these terms incorporate the concept that the light entering the water will be absorbed or scattered by particles, dissolved colored matter, and by the water itself. What can be noticed from the equation is that these terms are in the denominator of the Secchi disk equation. As the attenuation of light by dissolved colored matter or particles increases, the Secchi depth decreases. This inverse relationship between Secchi depth and particulates or color produces the typical hyperbolic curve when Secchi depth is plotted against chlorophyll or turbidity (Fig. 2.6).

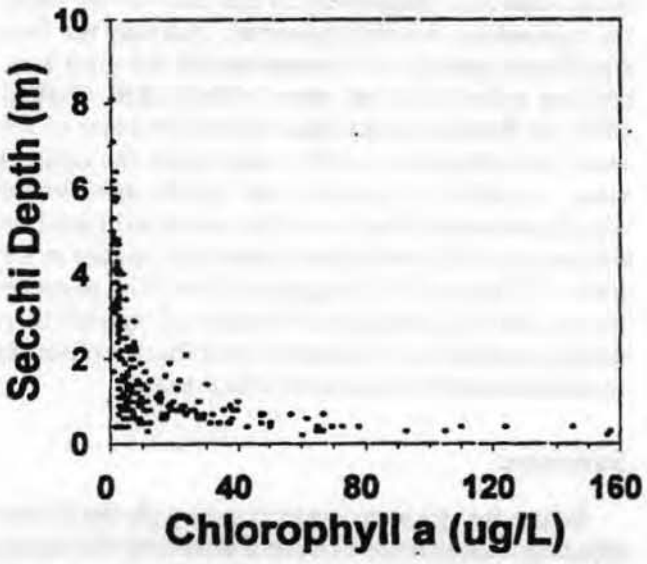


Figure 2.6.—The relationship between Secchi depth and chlorophyll *a* concentrations in Florida lakes. After Canfield and Hodgson (1983).

What is considered interference and what is considered an accurate Secchi disk reading depends heavily on the use of the Secchi depth data. If the intent of the measurement is simply to obtain transparency information, then Secchi depth can be used without objection. Preisendorfer (1986a) argued that a Secchi disk value should be considered as a simple visual index of the clarity of a body of water. He cites the work of Arone (1985) as an example of a legitimate use of Secchi data. Arone used 96,000 Secchi depths to produce an atlas of Secchi depths around about 50% of the world's coastlines. Such use of Secchi disk information makes no assumptions as to the cause of the varying transparencies. Only further studies with more sophisticated instrumentation and methods could reveal the causes of variation. On the other hand, the variation in Secchi depths provides the stimulus for further investigation.

Estimating Chlorophyll From Secchi Depth

The intent of most volunteer programs is not to measure transparency, but to use Secchi depth as a surrogate measure of algal chlorophyll or algal biomass, and subsequently, as an indicator of the trophic state. When Secchi depths are used as an estimator of other, unmeasured variables, additional potential interferences can become important. In theory, chlorophyll can be estimated from Secchi depth because it is a substance that attenuates light in the water column. Chlorophyll is also packaged in algal cells, and these cells absorb and scatter light.

One should be able to use Secchi disk data as a surrogate estimator of algal abundance, either by producing empirical relationships between Secchi depth and chlorophyll or by deriving the chlorophyll concentration based on the theoretical relationship between transparency and chlorophyll. In theory, both α, (beam attenuation coefficient) or K (vertical attenuation coefficient) can be partitioned into terms that attenuate and scatter light (attenuation and scattering by water, dissolved substances, non-chlorophyllous particulates, chlorophyll, etc.). However, usually only K is partitioned; the remaining non-partitioned variable, α, is assumed to be a constant function of the other (i.e., the term $(\alpha + K)$ becomes $K(1+\alpha/K)$). The K outside the parentheses in the term, $K(1+\alpha/K)$, is then partitioned into component parts and the variables inside the parentheses are considered constant.

The degree of partitioning of the terms can be quite detailed (Tilzer 1988), but often the vertical attenuation coefficient is partitioned into the attenuation and scattering of light by chlorophyll and a single term, K_w (Megard et al. 1980), that incorporates all other factors such as dissolved colored material, non-algal particulates, and water. The chlorophyll term is then further subdivided into a chlorophyll concentration term (Chl) and a specific chlorophyll attenuation term (K_c), producing the equation:

$$K = K_w + K_c Chl \qquad (2.5)$$

In this equation, K and K_w have the dimensions (1/meters), while K_c has the units of m^2/mg in order to complement chlorophyll concentration's units of mg/m^3. If we assume that α/K is constant, the general Secchi depth equation (2.2) would become,

$$z_{SD} = \frac{\ln (\tau C_o / C_R)}{K (1 + \alpha / K)} \qquad (2.6)$$

The vertical attenuation coefficient, K, could be partitioned to produce

$$z_{SD} = \frac{1}{K_w + K_c Chl} \frac{\ln (\tau C_o / C_R)}{(1 + \alpha / K)} \qquad (2.7)$$

If all the variables in the second set of brackets are assumed to be constant (or at least vary little), then this set of variables can be represented by a single term, L, (which should probably stand for "Leap of Faith") and the equation reduced and rearranged to produce the equation,

$$z_{SD} = \frac{L}{K_w + K_c Chl} \qquad (2.8)$$

This can be rearranged to produce an equation that allows the prediction of chlorophyll from Secchi depth:

$$Chl = \frac{L}{K_c} \frac{1}{z_{SD}} - \frac{K_w}{K_c} \qquad (2.9)$$

If K_c, K_w, and L were constant and known, then chlorophyll could be predicted from Secchi depth.

Since such knowledge of these variables is often difficult to obtain independently, usually an empirical relationship between Chl and SD or $1/z_{SD}$ is derived instead. For such an empirical equation to be useful, K_c, K_w, and all the variables in L must be constant or have relatively little variation within the data set. With data sets from single lakes over limited time periods, gathered with consistent methods, these assumptions may hold true. However, using these derived relationships over a larger group of lakes, or even over other time periods in the same lake, invites problems. Variations in the light attenuation of both chlorophyll and other substances will cause scatter in these relationships.

Non-Algal Attenuation

Light attenuation or scattering by any substance other than algal chlorophyll can cause errors in the estimation of chlorophyll from Secchi depth measurements. In Equation 2.5, these substances are lumped into K_w, the non-algal attenuation. Dissolved humic substances and non-algal particulates are common co-attenuators and scatterers of light. These substances, of course, increase K_w, but, because they also reflect and absorb light, they also change the "brightness" of the water and therefore affect C_o/C_T, and may also affect the relationship between α and K. In either case, L is affected as well as K_w. The attenuation of light by chlorophyll, K_c, is also not constant (Carlson 1980), appearing to co-vary with chlorophyll concentration.

Variation in K_c, K_w, and L results in increased scatter in the empirical relationship between chlorophyll and Secchi depth. In lakes where the dominant attenuators of light are non-algal particulates or dissolved color, the prediction of chlorophyll from Secchi depth becomes impossible. The problem is that, unless ancillary measurements of chlorophyll, color, or non-algal turbidity are made, it is impossible to determine which Secchi measurements are being affected by these interferences. Thus every Secchi depth value is suspect.

A common misunderstanding about these non-chlorophyllous attenuating substances centers around the idea that there is a threshold below which they become "unimportant." That is, below some value for these substances, the error produced by the interfering substance cannot be distinguished from the "normal" scatter in the Secchi depth-chlorophyll relationship. For example, in Fig. 2.7 the data of Canfield and Hodgson (1983) have been plotted to illustrate the deviation of chlorophyll predicted from Secchi disk from the actual chlorophyll concentrations as a function of the amount of dissolved color in the water. The predicted and actual chlorophylls have been transformed into the trophic state indices of Carlson (1977); the difference between the predicted chlorophyll (TSI(Secchi Depth)) and actual chlorophyll (TSI(Chl)) should be zero if there was no interference from color.

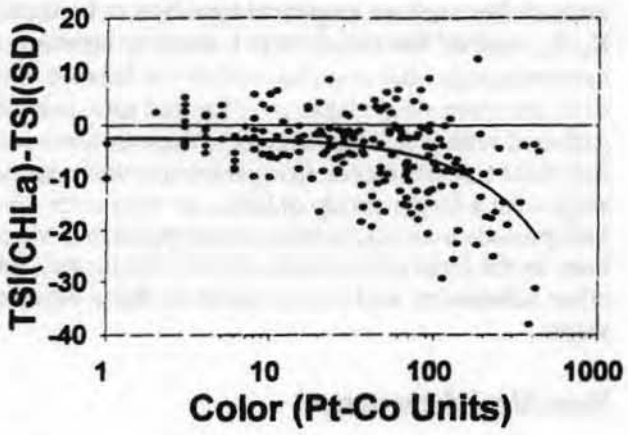


Figure 2.7.—A residual analysis of the effect of dissolved water color on the deviation of chlorophyll predicted by Secchi depth from the actual chlorophyll concentration. The data have been transformed using the trophic state index of Carlson (1977). Data from Canfield and Hodgson (1983).

The deviation of predicted from actual chlorophyll is evident at color values greater than 100 platinum-cobalt units, but the deviation begins at much lower values, perhaps as low as 10 platinum-cobalt units. Schloss (1988) also found that color values greater than 30 had a detectable affect on Secchi depth. Other interfering factors, such as non-algal turbidity, should produce similar increasing deviations in the chlorophyll-Secchi depth relationship.

Empirical methods cannot easily extract the chlorophyll attenuation from the interfering factors because of the difficulty of finding independent estimators of non-algal attenuation. Water color, turbidity, and inorganic suspended solids have been used with varying success as estimators of non-algal attenuation. A monitoring coordinator, therefore, should consider meaasuring color and turbidity or suspended solids in conjunction with Secchi depth if the objective to estimate chlorophyll. If this is the case, however, why not simply measure chlorophyll and skip the ancillary measurements?

The best reason for measuring variables other than Secchi depth is not to improve the prediction of chlorophyll from Secchi depth, but to aid in the estimation of the other light-attenuating factors. These factors may also play a role in the determination of the dynamics of the lake. For example, there is a relatively simple procedure to obtain estimates of K_w and K_c if chlorophyll information is gathered with Secchi depth. As demonstrated by Megard et al. (1980), if equation 2.9 is rearranged and K partitioned, the following equation would be obtained.

$$\frac{1}{z_{SD}}(L) = K_w + K_c Chl \qquad (2.10)$$

This equation assumes that L, K_w, and K_c are constant within the data set.

According to this equation, a plot of $1/z_{SD}$ against chlorophyll concentration should produce a straight line with a slope K_c and a Y-intercept of K_w (Fig. 2.8). The equation is particularly useful on seasonal data from a single lake. The evaluation of slope and intercept of the plot can give an estimate of the relative importance of non-chlorophyllous attenuating substances in water clarity. The intercept should change as the amount of non-chlorophyllous materials in the water varies. The slope, which reflects the light attenuation per unit chlorophyll, may change seasonally as different algal species become dominant.

The method appears to fail as non-algal attenuating materials become abundant. The slope of the line falls to zero as the intercept becomes larger, perhaps because of changes in L. There also seems to be a relationship between slope and intercept that is not predicted by theory (Carlson 1980). Despite possible limitations, if chlorophyll data are available, some interesting regional patterns or changes with trophic state may emerge if this procedure was done on each lake in the data set.

Walker (1982) used a different method used to estimate the amount of non-algal material in the water. Walker rearranged Equation 2.10 to produce

$$K_w = \frac{L}{z_{SD}} - K_c Chl \qquad (2.11)$$

Unlike the use of the Megard's partition equation given above, Walker's equation requires that K_c be

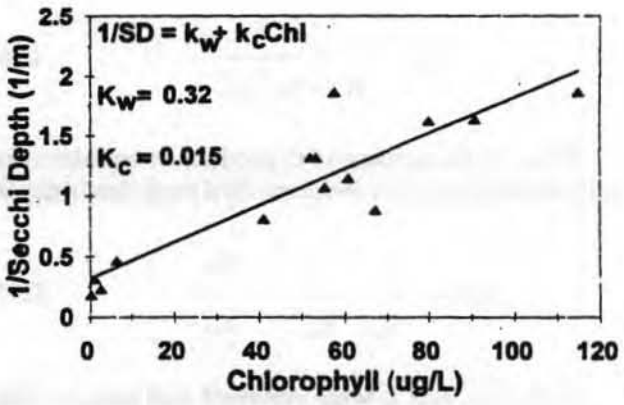


Figure 2.8. If the inverse of Secchi depth is plotted against chlorophyll, the amount of attenuation of non-algal substances will be reflected in the Y-intercept and the attenuation of light by chlorophyll in the slope of the line. Data from Halsteds Bay, Lake Minnetonka, Minnesota.

known and constant. Walker used a value of 0.025 m^2/mg for K_c, based on the slope of the maximum transparency per unit chlorophyll derived from his data set. However, K_c is known to vary and may even vary as a function of chlorophyll concentration (Carlson 1980). The equation may estimate the maximum potential amount of non-chlorophyll particulates, but may seriously overestimate the actual amount of particulates present.

Using Empirical Models

Sometimes program coordinators cannot avoid using empirical relationships to predict chlorophyll from Secchi depth values. Programs are often charged with determining the "trophic state" of lakes, but have only Secchi depth values available. In these cases, the coordinator must use empirical relationships between chlorophyll and Secchi depth. However, it is the responsibility of the coordinator to emphasize and evaluate the potential error involved in such a use of Secchi depth. The coordinator should use these empirical models with caution and with some knowledge of their limitations.

Empirical models work best in situations where chlorophyll is the dominant attenuating substance. In these cases, a regression of 1/SD against chlorophyll, might yield a useful predictive model. It is important to plot the data as well as derive a predictive equation. In many instances, this relationship can have considerable *heteroscedastcity*, that is, the variance increases with increases in chlorophyll. To minimize this effect, often the logarithm of Secchi depth is plotted against the logarithm of chlorophyll (Fig 2.9). Probably most empirical models use log-transformed data, and the plot of the data should produce a straight line.

If the scatter is not linear, then some variable other than Secchi depth or chlorophyll may be affecting the model. This can be seen in Fig. 2.7, where dissolved water color increases the scatter in the relationship. Residual analysis, the plotting the deviations of the actual data points from the predicted value against various other measured variables (e.g., color, inorganic suspended solids, turbidity, etc.), can be used to reveal if there are any other variables that should be considered and perhaps isolated. A form of residual analysis was used in Fig. 2.7. In this case the TSI(SD) is the estimated or predicted chlorophyll—which is compared to the TSI derived from the actual chlorophyll data, TSI(Chl).

Deviations of the predicted from the observed can be used to identify possible confounding variables. Once confounding variables have been identified, they can be isolated by grouping the Secchi depth data into categories. These might include natural lake versus reservoir, or into geographic regions, such as the

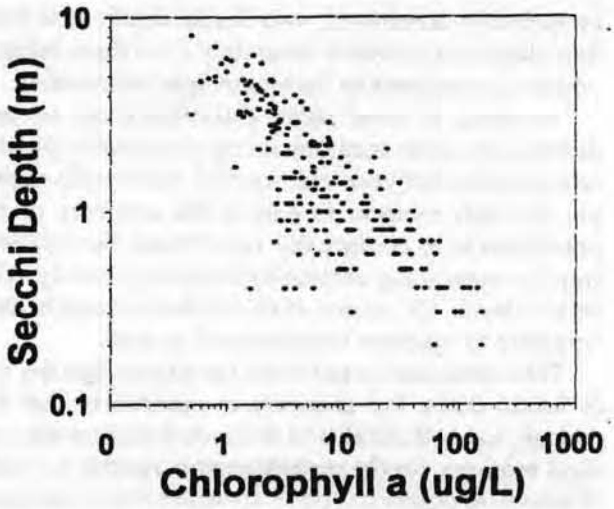


Figure 2.9. Empirical relationships between Secchi depth and chlorophyll are usually done using an empirical relationship based on a plot of the logarithm of Secchi depth against the lagarithm of chlorophyll. Data from Canfield and Hodgson (1983).

ecoregions (Fig. 2.10). These variables can also be incorporated into the predictive equation itself using multiple regression techniques. In the case of volunteer monitoring programs, the use of geographic or lake type categories might be most useful because they do not require ancillary variables.

A simple method for isolating highly turbid lakes is to use the color of the water, as measured by the volunteer, to distinguish between lakes having high non-algal turbidity. The Ohio NEFCO and the Ohio CLIP programs use a color strip to estimate the color of the water (LaBounty 1993). There is evidence from a number of northern Ohio lakes in the Ohio-NEFCO program that the relationship between chlorophyll and Secchi depth deviates sharply when the reported water color is brown. These brown-colored lakes may

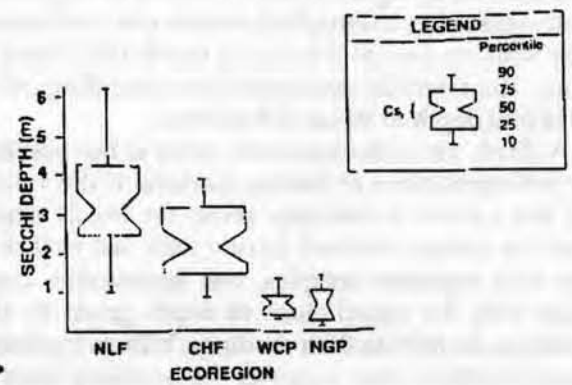


Figure 2.10.—Box plots of summer Secchi depth values of four ecoregions in Minnesota (After Heiskary, 1989).

be turbidity dominated, and Secchi depth data from these lakes are reported separately from those lakes in which algae appear to dominate light attenuation.

At times, in some lakes, chlorophyll can be predicted with some accuracy using empirically derived relationships between chlorophyll and Secchi depth, but the only method to ensure the accuracy of the prediction is to continually re-calibrate the relationship by measuring chlorophyll simultaneously with Secchi depth. Of course, if this is done, there is little necessity to measure transparency as well.

This discussion is not meant to discourage the use of Secchi disks, but is meant to emphasize that the primary use of the disk is to measure transparency, not algal biomass. Secchi measurements remain the base of volunteer diagnostic efforts because Secchi disks are inexpensive and simple to use. It may be necessary to use Secchi depth as a surrogate estimator of algal biomass in volunteer programs, but it should be done with considerable caution.

Other Considerations

Lowering Line

A number of programs use a marked line. Volunteers simply read the Secchi depth based on the markings on the line. The advantage of this method is simplicity, but there are several disadvantages to marking the line. A number of programs report shrinkage in the line. Shrinkage could introduce a significant error in the measurement. Volunteers should measure their rope at the beginning and end of each season to verify that the markings have not changed.

Another problem with a marked line is that the reporting interval used does not allow for the real accuracy that is possible with the Secchi depth. Measuring Secchi depth at intervals of even 1/4 foot can introduce significant errors in low transparency waters. This is illustrated in Figure 2.9, using the data of Canfield and Hodgson (1983). The plot of log Secchi depth versus log chlorophyll reveals ever increasing gaps with no data as the Secchi depth falls below 1 meter. The intervals correspond to a rounding off of the Secchi depth to 10 cm (3.9 inches).

A third, yet undocumented, error is the possible psychological effect of having markers. If the volunteer has *a priori* knowledge about the Secchi depth based on values obtained during their last visit or if they take replicate samples, this knowledge, combined with the visual clues of depth given by the markings, could bias their readings. Wilson (personal communication) also suggests that marking lines is very expensive.

The Ohio NEFCO program uses an unmarked line and a ruler or yardstick to measure depth. This technique requires the additional equipment of a measuring device and added time of measurement. However, the advantages of not having to worry about shrinkage, the reduced cost, and the elimination of statistical or possible psychological errors may outweigh the additional equipment and effort.

Sampling Protocol

Little mention is made about the nature and limitations of the observer in Secchi disk monitoring programs, probably because most monitoring programs are happy to have all the volunteers they can handle. However, the observer is an integral part of the Secchi depth measurement, and therefore the observer's abilities should be considered. There are little data available as to the effect of the observer's abilities or biases on Secchi depth measurements, but certainly their eyesight should be considered. None of the programs reviewed recommend that the volunteers have 20-20 corrected vision, although this would seem to be a minimal prerequisite for inclusion in the program.

It is also possible that each person might differ in what they consider to be the depth at which the disk disappears, meaning that the Secchi depth is, in part, a matter of judgment on the part of the observer. Carlson once took a class to a lake, and, with no more instruction than to measure the depth of disappearance of the disk, had them take three measurements of Secchi depth. The median percentage variation in Secchi depth of each student was very low (2.6% with a range of 0.8-9.4%). The range of median values found between students varied from 1.40 to 1.73 meters, however. It appeared that each student was able to replicate their own Secchi depth measurement quite well, but that depth varied considerably between observers. Perhaps the best way to achieve some semblance of uniformity between observers is to have careful training of the observers before and during the monitoring season.

When possible, the sampling location should be at least fifty percent greater than the Secchi depth so that the disk is viewed against the water background, not bottom-reflected light (Davies-Colley et al. (1993). If the disk touches the bottom before it disappears, the readings should be reported, but it should be recorded that the disk reached the bottom. These numbers are important because they contain information about the minimum Secchi depth on a given day, but they cannot be used in computing seasonal averages. The values can be used by either using the median Secchi depth values rather than averages (K. Reckhow, personal communication) or use a log-probit analysis technique to find the geometric mean of the data (Travis and Land 1990). Probit analysis assumes that the samples

were taken from the same lognormal probability distribution, and if all the data are plotted on a probit scale, they will lie in a straight line. The geometric mean is determined from the 50th percentile value.

Schloss (1988) found that if the Secchi depth was close to the lake bottom, sediments could affect the Secchi depth. Sediments could be stirred up by the anchor, bottom currents, or even the Secchi disk itself. Volunteers should be taught to minimize the disturbance of the bottom sediments prior to taking the Secchi readings. They should use a sufficiently long anchor line so that the boat moves away from the anchoring site and sediments disturbed by the anchor do not interfere with the readings. They should also not attempt to find the depth of the location prior to taking readings. In shallow lakes, entrainment of material from the bottom can significantly affect subsequent readings.

Methods Used by Programs in the Upper Midwest

The ease of using a Secchi disk, combined with citizen interest in water clarity and the relative low cost of buying or making a disk, makes the Secchi disk water clarity measurement the most popular activity undertaken by volunteer lake monitoring programs. Its popularity holds true in the Upper Midwest. Table 2.1 summarizes the Secchi disk materials and methods used in the statewide programs in Indiana, Illinois, Michigan, Ohio and Wisconsin.

Disk

The circular plate that is used as a Secchi disk can be made from virtually any material. An important attribute, however, is that it be heavy enough to be lowered into the water column with a minimal amount of wobble and to stabilize itself when the volunteer makes a reading. A disk that is too light has a tendency to move with wind and water currents. Most program managers have found that one to two pounds is an optimum weight for the Secchi disk assembly (Fig. 2.10).

The Illinois and Wisconsin Programs buy their Secchi disks from a manufacturer. The disks are constructed from sheet metal and have the alternating black and white quadrants painted on with a waterproof enamel paint. Officials with the Indiana, Michigan, Minnesota, Ohio monitoring programs have a more personal involvement in the construction of their Secchi disks. The Indiana Volunteer Lake Monitoring Program arranges for the Indiana University Physics Department Metals Shop to cut their disks from sheets of white PVC plastic. Students at the University School of Public and Environmental Affairs then paint the

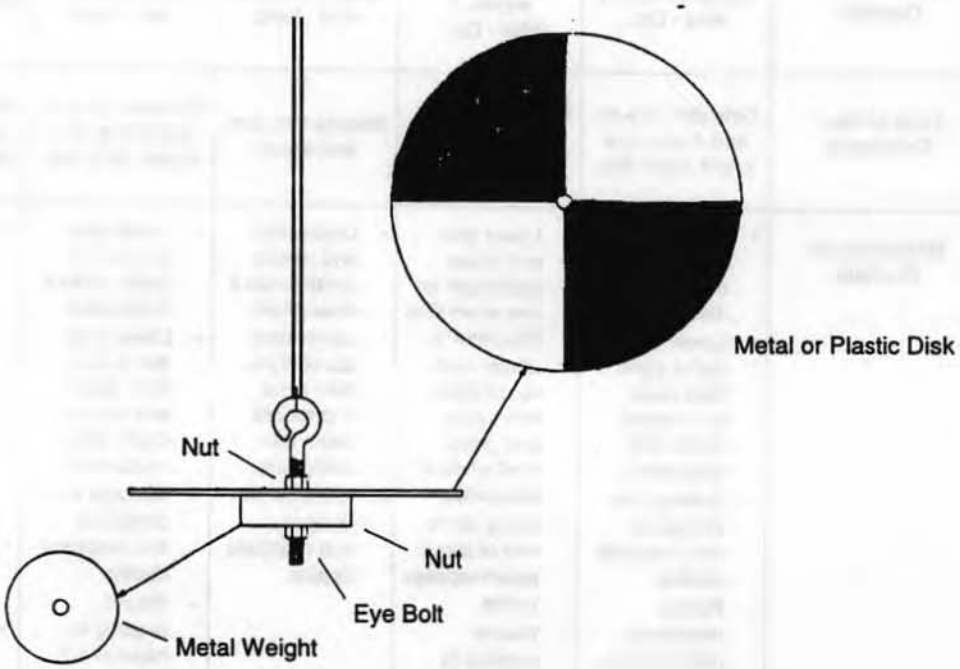


Figure 2.10—Components of a typical Secchi disk assembly includes a disk with alternating black and white quadrants, a weight, an eye bolt and a lowering line.

Table 2.1: Comparisons of Secchi disk materials and methods

		ILLINOIS	INDIANA	MICHIGAN	MINNESOTA	OHIO	WISCONSIN
EQUIPMENT	Secchi disk Materials	Metal plate, black & white enamel paint	White PVC, black enamel paint	White styrene, black silk screen ink	Metal plate, glossy white epoxy enamel paint	Metal plate, black & white enamel paint	Metal plate, black & white enamel paint
	Diameter/ Pattern	8 inch diameter 	8 inch diameter 	8 inch diameter 	8 inch diameter 	8 inch diameter 	8 inch diameter 
	Lowering Line / How Stored	Polypropylene cord / Cord wrapped around a dowel.	Dacron cord / Cord wrapped around a dowel.	Fiberglass measuring tape / Wound up in a reel.	Dacron cord / Disk is notched, cord is wrapped around it	Nylon cord / Cord wrapped around a cord wrap.	Polypropylene cord / Cord wrapped around a dowel.
	Units / Line Markings	Inches / Line marked in 2-inch intervals.	Feet / Line marked in 1 foot intervals.	Feet and Inches / Measuring tape	Feet / Line marked in 1/2 foot intervals.	Inches/ Line marked in 1 foot intervals to 10 feet, then 5 foot intervals.	Feet/ Line marked in 1 foot intervals.
	Assembled by / Cost per Unit	Purchase from manufacturer / $12 per unit	IU students / $12 per unit	ML&SA volunteers/ $10 per unit	MPCA staff / $10 per unit	State Park and CCC Camp staff / $10 per unit	Purchase from manufacturer / $12 per unit
LOCATION / TIME	Site(s) Location	Generally 3 sites, but some lakes have more and some less.	1 site, deepest section	1 site, deepest section	1 site, deepest section (additional sites optional)	1 site, deepest section (additional sites optional)	1 site, deepest section
	Frequency / Duration	Twice a month. / May - Oct.	Once every two weeks. / May - Oct.	Once a week. / April - Sept.	Once a week. / Jun. - Sept.	First and third weeks of month./ May - Oct.	Ideally once a week, bi-weekly acceptable, April - Nov. (Jul.-Aug. min.)
	Time of Day / Conditions	Between 10 a.m. and 4 p.m on a bright, calm day.	Between 10 a.m. and 4 p.m on a clear, calm day.	Between 10 a.m. and 3 p.m.	Between 10 a.m. and 3 p.m on a bright, calm day.	Between 10 a.m. and 4 p.m on a bright, calm day.	Between 10 a.m. and 4 p.m on a clear, calm day.
INSTRUCTIONS	Measurement Protocol	• Lower disk and record depth when it disappears. • Lower until out of sight, then raise and record depth disk reappears. • Average the disappear and reappear depths. • Round reading to nearest inch.	• Lower disk and place clothespin on line when disk disappears. • Lower until out of sight, then raise and grasp cord when it reappears. • Move pin to mid of disappear/reappear points. • Round reading to nearest 1/4 foot.	• Lower disk and record depth when it disappears. • Lower until out of sight, then raise and record depth disk reappears. • Average the disappear and reappear depths.	• Lower disk and record depth when it disappears. • Lower until out of sight, then raise and record depth disk reappears. • Average the disappear and reappear depths. • Round reading to nearest 1/2 foot.	• Lower disk and place pin on line when disk disappears. • Lower until out of sight, then raise and place 2nd clothespin on line when disk reappears. • Record mid disappear/ reappear. • Round reading to nearest 1/2 inch.	• Lower disk and place pin on line when disk disappears. • Lower until out of sight, then raise and grasp cord when it reappears. • Move pin to mid of disappear/ reappear. • Round reading to nearest 1/4 foot.

black quadrants on the white disks and add a weight, eye bolt and lowering line. The Ohio program uses metal disks that are stamped out at the Barkcamp State Park. Employees at the Civilian Conservation Corps Camp then paint on the alternating black and white quadrants. CLIP volunteers and staff complete the final assembly.

Volunteers of the Michigan Lake and Stream Association (ML&SA) make their Secchi disks at a home workshop. Using an eight inch diameter template, they mark out disk outlines on 4' by 8' sheets of white styrene plastic (about sixty disks per sheet). Center holes are drilled and the disks rough cut with a saber saw. They are then rounded out using a table mounted router. A silk screen is then made of the Secchi disk pattern. Using a frame and a special silk screen ink that adheres to plastic, volunteers print the black quadrants on the top side of the disk and program information on the bottom side.

For the final assembly, volunteers bend straight ladder bolts into eyebolts for the attachment of the lowering line. They also make Secchi disk weights by melting scrap lead and using a muffin tin as a mold. A hole is drilled in the weight and the components are attached. Lastly, ML&SA volunteers cut out and sew canvas bags (with draw strings) for convenient storage of the Secchi disk.

As mentioned above, the Minnesota Citizens Lake-Monitoring Program uses an eight inch all white Secchi disk. The disks are metal, cut and spray painted with white epoxy enamel paint by a contractor. Program officials complete the Secchi disk kit by including a marked cord attached to an eyebolt and a packet containing washers and hex nuts. The volunteer completes the assembly at home.

Lowering Line

The type of line used to lower the Secchi disk into the water is an important consideration because the line doubles as a ruler for measuring depth. It is crucial that markings hold true through many months of service. The line must be made of a material that does not stretch or contract over time.

Through experience program coordinators have learned that most natural fiber lines, as well as nylon lines, have a tendency to shrink if exposed to repeated wetting and drying. For this reason, the volunteers of the Illinois, Indiana, Minnesota and Wisconsin program use a dacron or polypropylene cord as their lowering line. Michigan volunteers, on the other hand, use a fiberglass tape as their lowering line. The Ohio program people currently use a nylon line, but plan to investigate the use of another material for future seasons because of the shrinkage problem.

Storage of a Secchi disk lowering line when not in use is a practical problem. This is especially true for programs that required volunteers to take a lake depth measurement as well as a Secchi disk measurement at their sample site. In many lakes this means volunteers need a line of forty feet or more in length. Illinois, Indiana, Ohio and Wisconsin program officials provide volunteers with a dowel or similar implement to wrap and store their line. Michigan volunteers are provided a reel system to wind up and store their fiberglass tape. The Minnesota Program takes a unique approach to the line storage problem. Their Secchi disks have notches cut at opposite ends of the disk. The line can then be neatly wrapped around the disk by nestling the line in the notches.

Measurement Units

Two important questions must be answered when designing a Secchi disk program:

1. What units of measurement will be used for making and reporting Secchi depth?; and
2. What measurement intervals will appear on the lowering line?

The Illinois Program uses inches as its primary unit of measurement. This small unit was selected because of the low clarity of most Illinois lakes and reservoirs. Officials mark the lowering line at two inch intervals with waterproof ink and volunteers record their Secchi disk measurements to the nearest inch. In contrast, the Wisconsin and Indiana Programs use feet as their primary unit of measurement. Lowering lines are marked at one foot intervals and volunteers are instructed to record their readings to the nearest one quarter foot. Minnesota officials mark their lines at one-half foot intervals. To assist volunteers with determining length, the foot intervals are marked in black and the half-foot intervals in red. Five foot multiples are doubled marked in black. Volunteers are asked to report measurements to the nearest one-half foot.

Michigan officials have taken a novel approach to making Secchi disk measurements. Instead of using a calibrated cord like the other programs, they instead rig their Secchi disk assembly with an actual fifty-foot fiberglass measuring tape. The tape is housed in an open reel that allows the volunteer to conveniently "reel" the disk up and down the water column. The measuring tapes attached to the disks distributed to Michigan volunteers in 1991 and 1992 were in traditional feet and inches. For the 1993 season, however, program officials are contemplating changing to a tape marked in feet and tenths of feet.

Ohio officials, on the other hand, have raised a concern about the practice of marking the lowering line. They theorize that some volunteers may have a

tendency to preconceive Secchi disk measurements if they watch and mentally count line markings while lowering the disk in the water column. Thus, a bias (or self-fulfilling prophecy) could be introduced if, for example, the volunteer wants to set a "record" reading or "beat" the clarity of the lake in the next town.

For this reason, Ohio officials mark their lines at one foot intervals but require their volunteers to report their Secchi disk readings to the nearest half inch. Consequently, the volunteer must flag the disappearance / reappearance points on the line, pull the disk out of the water, lay the line against a twelve inch ruler (using the foot-long interval marks as a guide), and then measure a reading to the nearest half-inch. This procedure thus eliminates the possibility of determining the measurement while the disk is being lowered into the water column.

Illinois Volunteer Lake Monitoring Program

Volunteers participating in the Illinois Volunteer Lake Monitoring Program receive individual instruction on how to take a Secchi disk reading. Program trainers travel to the volunteer's lake and teach them in their sampling boat. During the session volunteers also learn how to find their sites and how to conduct additional field observations.

Listed below are the instructions given to Illinois program volunteers.

- Anchor the boat at the sampling site and remove sunglasses;
- Lower the Secchi disk on the shaded side of the boat until the point it disappears from view and note that depth;
- Lower the disk farther and bring it back up until it just reappears; and
- Take an average of the two readings and round it to the nearest inch.

Indiana Volunteer Lake Monitoring Program

Volunteers in the Indiana Volunteer Lake Monitoring Program are trained by Clean Lakes Program staff at the annual Indiana Lake Management Conference or in group training sessions held around the state. Volunteers also receive a training manual entitled, *Volunteer Lake Monitoring Handbook*. This training manual presents a background of the program, a checklist of needed items for monitoring, and specific step-by-step instructions for taking a Secchi disk reading.

Listed below are the instructions given to Indiana program volunteers.

- Use the map of your lake and its marked sampling site and proceed to the site;
- Anchor the boat at the sample site, remove your sunglasses and unwind the Secchi disk rope from the dowel;
- Lean over the shady side of the boat and slowly lower the disk into the water until it no longer can be seen;
- Mark the rope at the water level with a clothespin;
- Lower the disk a few more feet into the water then slowly raise the disk. When the disk reappears, mark the rope at the water level with your fingers;
- Form a loop between the clothespin and your fingers, slide the clothespin to the center of the loop and haul the disk back into the boat; and
- Carefully count the number of feet from the disk until you reach the clothespin. Round off to the nearest one quarter foot.

Michigan Inland Lake Monitoring Program

Volunteers in the Michigan Program have an opportunity to receive Secchi disk training if they attend a regional meeting or the annual meeting of the Michigan Lake and Stream Associations (ML&SA). However, attendance at a training session is not required to participate in the Michigan Program. All volunteers receive a set of simple instructions with their Secchi disk.

Listed below are the instructions given to Michigan program volunteers.

- Anchor the boat before measuring transparency to make sure that the Secchi disk is observed straight down;
- Lower the disk into the water on the shaded side of the boat;
- Note the depth at which the disk disappears;
- Lower the disk farther and then raise and note the depth at which it reappears; and
- Take the average of these two readings.

Minnesota Citizens Lake-Monitoring Program

Volunteers in the Minnesota Citizens Lake-Monitoring Program receive their Secchi disk training via a detailed instruction sheet sent in the mail with their Secchi disk. No personal individual or group training is conducted. However, volunteers are encouraged to call program officials if they have any questions. Also it should be noted that program officials carefully check over data that is sent in by the volunteers. If an error or inconsistency is noted, an official will call the volunteer and conduct "over-the phone" training.

Listed below are the instructions given to Minnesota program volunteers.

- Lower the Secchi disk into the lake, on the shaded side of the boat, until the disk just disappears from view. (If the volunteer can see

the disk on the lake bottom and cannot find deeper water, they are requested to indicate this on the data sheet);
- Note the disk's depth by way of the marked cord;
- Lower the disk a bit farther and then raise it until it just reappears. Note this depth;
- Average the two depths to the nearest 1/2 foot to get the Secchi disk transparency reading.

Volunteers in the Minnesota program are also instructed to remove sunglasses while making a Secchi disk reading. They are also advised that if they wear photo-gradient prescription eyeglasses, they should try to prevent them from darkening by wearing a hat or visor with a wide brim. Also, if the lake is choppy, try and take the reading from the stern of the boat. Volunteers are further advised that they may find their lake is exceptionally clear and that the rope is not long enough. If this is the case, they are instructed to add more line but to be careful when marking it at half-foot and foot intervals. If the markings become indistinct, they can be re-marked with a waterproof felt pen or with yarn threaded through the line. Volunteers are also advised that the disk will give longer and better service if it is kept clean and protected from scratches.

Ohio Citizen Lake Improvement Program

Ohio volunteers receive instructions on Secchi disk measurement protocol at one of several training workshops held in April throughout the state. In addition they receive a detailed instruction sheet entitled *Standard Operating Procedures*. This procedures document contains a check list of needed equipment and supplies, describes how to find the sample site using the triangulation method, and lists step-by-step instructions for taking the Secchi disk measurement.

Listed below are the instructions given to Ohio program volunteers.

- At the monitoring site, anchor the front and, if possible, the back of the boat to prevent drifting. Be careful not to disturb the sediments when anchoring since this could cloud the water and interfere with the disk reading;
- Once you are properly anchored at the monitoring site, go to the shady side of the boat. If you are wearing sunglasses, remove them;
- Lower the Secchi disk into the water until the disk just disappears from sight. Mark the rope at the water level with a clothespin;
- Lower the disk about two feet, then slowly raise the disk up until it reappears. Mark the rope at the water level with a clothespin; and
- To find the Secchi disk depth, form a loop with the two clothespins and locate the center of the loop. Measure the distance from the center of the loop to the Secchi disk. This is the Secchi disk depth. Record the measurement to the nearest half-inch.

Wisconsin Self-Help Lake Monitoring Program

Most volunteers in the Wisconsin Self-Help Lake Monitoring Program receive individual instruction by Wisconsin Department of Natural Resources (WDNR) lake specialists at their own lake. Alternatively, in some areas, group sessions are held at a centrally located lake. Volunteers in the Wisconsin Program receive a twenty-page instruction manual entitled, *Self-Help Lake Monitoring Handbook*. This document provides a background of the Wisconsin Program and explains the purpose of the Secchi disk measurement. The handbook also discusses when to monitor, why data may vary over the season, and the value of water color and lake level information. Lastly, it provides a checklist of equipment and supplies needed for monitoring and illustrated step-by-step instructions for making and recording a Secchi disk measurement.

Listed below are the instructions given to Wisconsin program volunteers.

- Use the map of your lake and its marked sampling site and proceed to the site.
- Anchor the boat at the sample site. Remove your sunglasses. Unwind the Secchi disk rope from the dowel.
- Lean over the shady side of the boat and slowly lower the disk into the water until it no longer can be seen.
- Mark the rope at the water level with a clothespin.
- Lower the disk a few more feet into the water. Raise the disk. When the disk reappears, mark the rope at the water level with your fingers.
- Form a loop between the clothespin and your fingers. Slide the clothespin to the center of the loop. Haul the disk back into the boat.
- Carefully count the number of feet from the disk until you reach the clothespin. Round off your measurement to the nearest one-quarter foot.

Recommendations

Based on the theory discussed above and on the methods, observations, and comments by existing programs, and the experience of the authors, the following recommendations are made.

Disk

It is recommended that the 20 cm disk be used. Although Davies-Colley (1988) recommends that the size of the disk should be changed as a function of the transparency of the water, there is insufficient evidence that the error would be sufficiently great to warrant the expense and possible volunteer error associated with switching disks.

It is recommended that a black and white quadrant disk be used. The all-white disk is historically correct and continues to be used in marine environments and in some volunteer programs. The black and white disk should give a somewhat smaller Secchi depth value than the all-white disk because the white surface area is smaller. However, the alternating black and white quadrants may reduce measurement variability, especially in water with a "bright" background.

The white portion of the disk should have a smooth, matte finish. The black portion should also be matte, because of its role as a total light-absorbing surface. The same paints should be used as more disks are made, so that the reflectance remains constant. Volunteers should keep the disk clean and free of scratches.

Lowering Line

If a marked lowering line is used, the line should be checked periodically for shrinkage. Volunteers should measure their rope at the beginning and end of each season to verify that the markings have not changed.

Volunteers should report their findings to the greatest accuracy possible, such as one centimeter or one inch. Values should not be rounded off to less than this. The use of an unmarked line should be considered.

Protocol

Careful training is necessary to assure the coordinator that the volunteers are reading the disk correctly and accurately. When possible, the sampling location should be at least fifty percent greater than the Secchi depth so that the disk is viewed against the water background, not bottom-reflected light.

Readings in which the disk touches the bottom should be reported, but it should be recorded that the disk reached the bottom. The values should not be used in computing seasonal averages unless some estimation technique is used.

A Secchi depth that is close to the bottom should also be considered suspect because of the possibility of the stirring of bottom sediments. The volunteers should be taught to minimize the disturbance of the bottom sediments prior to taking the Secchi readings. Use a sufficiently long anchor line so that the boat moves sufficiently away from the anchoring site and sediments disturbed by the anchor do not interfere with the readings. Do not attempt to find the depth of the location before taking readings; in shallow lakes, entrainment of material from the bottom can significantly affect subsequent readings.

Although theory suggests that every effort should be made to decrease errors caused by glare from the surface of the water, the use of sunglasses is not recommended. Lowering the disk on the shaded side of the boat to reduce glare adds additional errors, depending on whether the disk or the water column is in the shadow of the boat. The glare on the sunny side of the boat may also increase error as well. The use of the viewscope is still controversial, but its use should be explored.

Summary

The Secchi disk is and should be the cornerstone of volunteer lake monitoring programs. It is inexpensive and provides useful data. However, it does have a number of technical problems that need to be addressed. Many of the problems can be minimized by standardizing the equipment and carefully training the volunteers. Problems of interpretation generally arise when the transparency data is used for purposes for which the Secchi disk was never intended.

The Secchi disk measurement is subject to numerous interferences related to non-algal or non-chlorophyllous materials in the water. Although empirical relationships can be established in some lakes and regions relating Secchi depth to algal chlorophyll, these relationships can change seasonally and between lakes. Coordinators should use these relationships with caution and re-calibrate the relationships often.

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3.
Chlorophyll

Chlorophyll is the green molecule in plant cells that carries out the bulk of energy fixation in the process of photosynthesis. Besides its importance in photosynthesis, chlorophyll is probably the most-often used estimator of algal biomass in lakes and streams, at least in North America. Its popularity results from several considerations. It is a measure of algal biomass that is relatively unaffected by non-algal particles such as bacteria or detritus. It is also a fairly accurate measure of algal weight and volume. Finally, it acts as an empirical link between nutrient concentration and a number of important biological phenomena in lakes and reservoirs.

Chlorophyll is also relatively easy to measure. This facility of measurement contributes to its popularity, but the resulting values are far more ambiguous than most are willing to admit. Unlike other chemical tests, there is no standard chlorophyll nor a method for generating a standard curve, thus making the measurement susceptible to instrument error as well as to errors in collection and analysis. When a sample is filtered and extracted for chlorophyll analysis, it unfortunately contains a large number of pigments other than chlorophyll *a*, the primary pigment of interest in monitoring programs. The absorbencies of these other pigments can intrude into the wavelengths used in the spectrophotometric or fluorometric determination of chlorophyll *a*. Considerable differences occur between methods, each of which yielding different results.

Chlorophyll itself is not a single molecule but a family of related molecules, designated chlorophyll *a*, *b*, *c*, and *d*. Chlorophyll *d* is found only in marine red algae, but chlorophylls *b* and *c* are common in fresh water. The molecular structure of chlorophylls *a* and *b* consists of a ring-like structure called a *porphyrin* and a long organic phytol "tail." In the center of the porphyrin ring is a magnesium molecule (Fig. 3.1). Chlorophyll *c* lacks the phytol chain.

The relative concentrations within the cell of these chlorophylls vary with the species of algae, but chlorophyll *a* is dominant in all the eukaryotic algae and the prokaryotic blue-green algae (cyanobacteria). Other pigments are also present in algal cells. These are the *carotenes* and the *xanthophylls*. In the cyanobacteria,

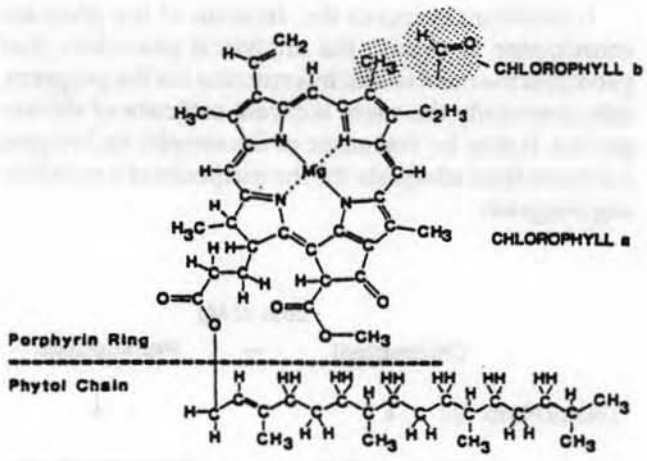

Figure 3.1—The chlorophyll *a* molecule, consisting of a porphyrin ring, a chelated magnesium molecule in the ring, and a long hydrocarbon (phytol) "tail." Chlorophyll *b* is the same molecule with the shaded groups missing. (From Weber *et al.* 1986.)

water-soluble *phycobiliproteins* are the predominant accessory pigment, giving the group their characteristic blue-green or red color. Besides the algal pigments, some bacteria are also pigmented with a series of *bacteriochlorophylls*.

Besides the naturally occurring pigments found in algal cells, a filtered sample will also contain colored degradation products of these pigments. When algal chlorophyll degrades, it forms a series of degradation products, the nature of which depends on what part of the molecule is affected. As a chlorophyll molecule degrades, the initial step is either the loss of the magnesium from the center of the molecule or the loss of the phytol tail. The former pathway results in the formation of the molecule phaeophytin; in the latter pathway, the resulting molecule is termed a *chlorophyllide*.

The degradation scheme is shown in Fig. 3.2. Further degradation of either the phaeophytin or the chlorophyllide produces a molecule termed a *phaeophorbide*: phaeophytin is degraded by the loss of the phytol tail and a chlorophyllide loses its magnesium ion. When a chlorophyll molecule breaks down, a number of distinct phaeophytins, chlorophyllides, and phaeophorbides will be produced, depending on the parent molecule. Some of these breakdown products are not easily separable spectrophotometrically from their parent molecule, producing falsely high absorbances and subsequent erroneous values for the living chlorophylls.

Despite its seeming simplicity in the analysis of chlorophyll, the validity of its results depends on whether these pigments interfere with the measurement of chlorophyll *a*. Almost every choice of analytical method addresses specific interferences, yet ignores others.

It therefore becomes the decision of the program coordinator to choose the analytical procedure that produces the most useful information for the program, not necessarily the most accurate estimate of chlorophyll *a*. It may be that some of the simpler techniques are more than adequate for the purposes of a monitoring program.

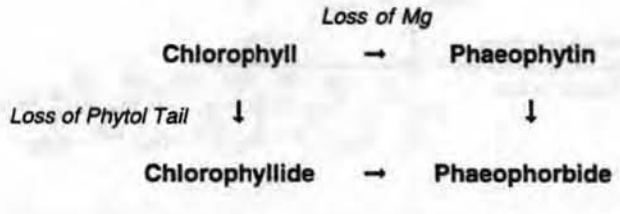

Figure 3.2—The degradation pathways of chlorophyll

Collection and Filtration

By some means, the volunteer must gather a sample of water. Some programs use a hose sampler, others use a water sampling bottle. Still others simply lower a sample container over the side of the boat. The means of collection will vary, based on the available equipment, the objectives of the study, and the opinion of the investigator as to what constitutes the best sampling technique. Sampling techniques will be discussed in more detail in Chapter 6.

Once the sample is taken, it is usually filtered and preserved until delivered to the laboratory for analysis. An alternative to filtering, preservation, and storage would be to immediately deliver the whole water sample to the laboratory. Herve and Heinonen (1982) suggest that whole-water samples stored at 4°C in the dark can be kept up to 1 day without significant degradation of chlorophyll. Weber et al. (1986) found no change in refrigerated samples over 18 days, but if the samples were left at room temperature (20°C), 50% of the chlorophyll was lost in 5 days.

Filtration is usually accomplished in volunteer monitoring programs using a filtration funnel and hand-held suction pump. This system allows the volunteer to filter large amounts of water in a relatively short time. The volunteer can also see how much algal material is being collected on the filter, and therefore judge when it is sufficiently green. This filtration technique does have the problem that the volunteer must measure the water in a separate, graduated container and must handle the filter, both before and after filtration. The volunteer must be trained to read a meniscus correctly and to handle the filters without touching them with the fingers.

The Ohio-NEFCO program uses a 25 mm in-line Swinney filter holder instead of a filter funnel. The glass fiber filter is placed into the filter holder by the coordinating laboratory prior to distribution to the volunteers. The volunteers are given a 60 ml plastic syringe equipped with a latex rubber hose and a 3-way valve. After the sample is brought into the boat, the volunteer places the tube into the sampler and draws 50 ml of sample into the syringe. The sample is then gently pushed through the filter positioned on the output end of the 3-way valve. Pulling back on the plunger switches the valve back into input mode and a second 50 ml of sample is drawn up into the syringe.

This alternation of drawing the sample and pushing it through the filter is done until an appropriate amount of algae has been filtered. When finished, the volunteer puts the exit end of the filter holder on the input tube and draws any remaining water out of the sampler. The entire sampler is then wrapped in foil, labeled, and stored in the freezer until picked up for analysis. The volunteer never touches or manipulates

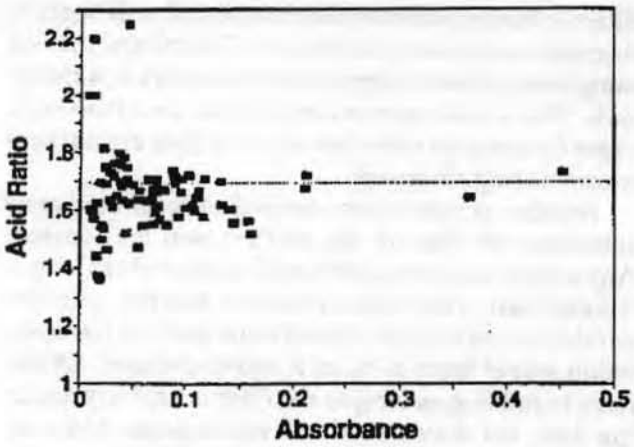

Figure 3.3—The relationship between the acid ratio of chlorophyll and the absorbance of the extract.

the filter. The pushing of the water through the filter, rather than using a gentle suction, may cause increased cell breakage, but this has not been studied. Care must also be taken to ensure all the water is removed.

The amount of algae filtered by the volunteer is important. There appears to be more variability in the acid ratio at low absorbances (Fig. 3.3) that may contribute to error in chlorophyll *a* values.* This variability may be a result of too much acidity, causing phaeophytin to be degraded to phaeophorbide (Hallegraeff 1976) or an analytical error associated with low chlorophyll concentrations. Standard Methods (APHA 1989) recommends an optical density of 0.1 to 1.0 at 664 nm if the trichromatic method is used. To avoid this problem, some programs instruct volunteers to filter until the paper is slightly green.

* The acid ratio is the ratio of unacidified to acidified absorbances. It is an important component in the calculation of chlorophyll *a* in some procedures.

The Florida program advises the volunteers to filter 100 ml of water for each foot of Secchi depth. The Ohio-NEFCO, Wisconsin, and Indiana programs developed a relationship between Secchi transparency and the amount of water to be filtered (Table 3.1). The Ohio-NEFCO table works reasonably well except in reservoirs having high non-algal turbidity. In that case, the amount of algae filtered is insufficient.

Choice of Filter

There are differences of opinion as to what type of filter to use. Three factors have been considered: retention of particles, efficiency of extraction, and cost. Membrane filters such as Millipore HA or Gelman retain more particles (Lenz and Fritsche 1980), but are more subject to clogging than are glass fiber filters.

This can mean that the volunteer might either have to filter less water or have to use very long filtration times. It also means that if a volunteer is faced with a clogged filter, they have to make a decision how to proceed. They can either start over, continue filtering, or, as is done in the Wisconsin program, pour the sample into another container, change the filter, and resume filtering.

Since Phinney and Yentsch (1985) suggest that major retention differences between membrane and glass fiber filters disappear above chlorophyll concentrations of 1 µg/L, glass fiber filters would seem to be adequate for most inland waters. Prepas et al. (1988) found no difference in chlorophyll concentrations collected on either Whatman GF/F (median retention size of 0.7 um) and GF/C (median retention size of approximately 0.2 um) glass fiber filters in concentrations ranging from 2 - 175 µg/L.

In oligotrophic waters, the choice of filter may be of more concern. Glass fiber filters have advantages be-

Table 3.1.—The relationship between Secchi depth and the amount of water filtered used in the Wisconsin and Indiana lake monitoring programs (Betz, et al. 1992) and in the Ohio-NEFCO program.

WISCONSIN & INDIANA		OHIO - NEFCO	
Secchi Depth (Ft)	Volume Filtered (ml)	Secchi Depth (Ft)	Volume Filtered (ml)
<1.0	50		
1.0 - 1.5	100		
>1.5 - 2.25	200	<2.0	100
>2.25 - 3.25	300		
>3.25 - 6.0	500	2.1 - 4.0	200
>6.0 - 9.75	800	4.1 - 8.0	300
>9.75 - 16.5	1,000	>8.0	400
>16.5	1,500		

cause they are less expensive than membrane filters and the glass fibers aid in the homogenization of cells during grinding. The membrane filters can be ground in a tissue grinder, but not as efficiently as glass fiber filters. Unless the superior extracting powers of a solvent such as methanol is used with membrane filters, the advantages of superior retention of smaller particles by membrane filters might be lost because of a lower amount of extracted chlorophyll.

Marker et al. (1980) listed the following reasons for using a Whatman GF/C glass fiber filter:

1. They are very efficient for chlorophyll retention in most situations and sometimes even better than membrane filters.
2. They filter much faster and do not rapidly clog.
3. On centrifugation there is no turbidity in acetone or methanol extracts.
4. They act as an excellent abrasive material to aid cell breakage during grinding.
5. For comparisons between various chemical variables, it is essential that the filters used for the determinations retain the same size fractions of particulate material. Most investigators now use glass fiber filters for these determinations as well as for particulate organic carbon.

Some methods suggest the addition of $MgCO_3$ to retard degradation and to enhance filtration efficiency. Some studies have found that its addition has no significant effect (Lenz and Fritsch 1980), and it may absorb pigments (Daley et al. 1973; Weber et al. 1986). If it is used with glass fiber filters, it could be used before filtration to decrease the pore size of the filter.

Preservation of the Chlorophylls

Once the chlorophylls are on the filter, they become highly susceptible to degradation as the cells die and decompose. They also become increasingly easy to degrade when exposed to light and even moderate temperatures. Some method must be used to keep the pigments from degrading. The problem is compounded for volunteer programs because the samples have to be transported to the laboratory for analysis. A simple mailing of the samples would be desirable, but there is the real possibility of degradation of the samples during the process.

The simplest method of preservation apparently is to freeze the samples. Several authors report that frozen samples showed no significant degradation even after 6 months (Lenz and Fritsche 1980). Jones and Lee (1982), however, mention that they have encountered problems with freezing. They recommend that samples should not be frozen unless investigation has shown that results from frozen samples are comparable to those from fresh samples. A problem with freezing the filters is that apparently the chlorophyll will begin to degrade as soon as it is unfrozen. This means that the samples must be brought to the laboratory in a frozen state. This would seem to preclude the use of the mails to get the samples to the laboratory if they are not kept frozen during shipment.

Another preservation method is to immediately submerse the filter in the solvent, seal and darken. Apparently the chlorophyll will not degrade as long as it is kept dark. This means, however, that the volunteer would have to be given the solvents and that transportation would have to be of a liquid chemical. Others have found that as long as the filter is kept dry and in the dark, the chlorophyll will not degrade. More experimentation seems to be necessary before an adequate preservation technique can be recommended.

Laboratory Methods

Several methods for chlorophyll analysis are available. The methods are described in Standard Methods (AHPA 1991), and the methods will not be discussed in detail here. However, there is a great deal of confusion about which method should be used in limnological investigations, and this confusion has resulted in a number of different methods being used by various programs.

Unfortunately, although all of these methods report their results as chlorophyll *a*, there is little evidence that the numbers derived by each method are necessarily similar. Because monitoring programs imply that the numbers generated are accurate as well as precise, the choice of a technique is important. A little background about chlorophyll analysis might help clarify the differences between the various chlorophyll methodologies used.

Homogenization and the Choice of Solvents

Homogenization by grinding of the filter enhances the rupture of the algal cells and increases extraction efficiency of the solvent. Homogenization is an absolute necessity with an acetone solvent, but some have found that other extractants such as ethanol or methanol apparently do not need grinding to extract all the chlorophyll (Sartory and Grobbelaar 1984). Others, however, have found that even methanol extractants do not extract as well without grinding. These other solvents are more efficient than acetone at extracting pigments from some green and blue-green algal cells. Methanol, however, is reported to be more toxic. Membrane filters can be ground but they lack the abrasiveness to produce a good extraction, and their extraction efficiencies are lowered (Long and Cooke 1971).

Spectrophotometric Techniques

Spectrophotometric analysis of chlorophyll pigments was developed in the 1930's and 1940's (Weber et al. 1986). Richards with Thompson (1952) introduced a trichromatic technique that was supposed to measure chlorophylls *a*, *b*, and *c*. Trichromatic equations attempted to remove interferences of the other chlorophylls at the maximum absorption wavelength for each chlorophyll.

Since Richards and Thompson, a number of modifications have been made to these equations which purportedly produce better estimates of the chlorophylls (Parsons and Strickland 1963; UNESCO 1966; Jeffrey and Humphrey 1975). When these equations are compared with concentrations of chlorophyll obtained using physical separation techniques such as HPLC, paper or thin-layer chromatography, it is found that the degree of correspondence is low.

Apparently the trichromatic equations are no substitute for physical separation techniques. In addition, these equations do not deal with the degradation products of chlorophyll. The trichromatic "chlorophyll *a*" is better presented as chlorophyll *a* minus most of the interference of other chlorophylls but including all degradation products that have absorbencies at the primary wavelength of chlorophyll *a*. These multiple chlorophyll equations have not been particularly successful, but are still used in oceanographic research, where degradation products are less of a problem.

Lorenzen (1967) and Moss (1967) introduced an acidification step in a monochromatic method to circumvent the interference by chlorophyll degradation products. When chlorophylls are acidified, the magnesium ion is lost from the porphyrin ring, resulting in the production of phaeophytin. Lorenzen (1967) produced equations capitalizing on the fact that the ratio of the absorbance of pure chlorophyll *a* after acidification to that before was 1.7. If the sample contained pure phaeophytin, then the absorbance would not change, and the ratio would be 1.0. Acid ratios between 1.0 and 1.7 indicate the amount of degradation products in the sample, and the estimate of chlorophyll could thus be corrected.

In natural waters, the acid ratio, and therefore the resulting estimate of chlorophyll *a*, varies considerably depending on, not only the relative concentrations of chlorophyll *a* and phaeophytin, but also the concentrations and behavior after acidification of chlorophyll *b*, phaeophorbides, chlorophyllides, bacteriochlorophylls, and perhaps even phycobiliprotein pigments. Numerous modifications have been made to this technique, including changing the normality of the acid, the absorbance wavelengths, the time between acidification and the reading of the value, and the extracting solvent. Despite major modifications in the technique, the resultant value is always called chlorophyll *a*, even though each methodological change alters the estimated chlorophyll *a* value. For example, other pigments, particularly chlorophyllide *a*, cannot be spectrophotometrically distinguished from chlorophyll *a*. These magnesium-containing degradation products of chlorophyll can comprise a significant proportion of the total pigments. Hallegraeff (1976) found magnesium-containing degradation products, on some dates, to comprise over 50% of what would have been estimated as chlorophyll *a*.

Hallegraeff (1976), as well as a number of other authors, emphasize that the acidification technique really eliminates only the interference of non-magnesium-containing pigments. Remember also that the trichromatic technique supposedly produced more accurate chlorophyll *a* estimates by removing interferences from other chlorophylls. The phaeo-pigment technique does not correct for these interfering pigments, but will incorporate the change in absorption of these pigments when they degrade upon acidification.

Spectrophotometrically determined "chlorophyll *a*," whether determined using trichromatic equations, or using the pigment correction is really an operationally defined term, whose meaning and values change with each alteration of the technique. Numerous authors emphatically state that the only method for measuring chlorophyll *a* accurately is using some separation procedure such as high pressure liquid chromatography (HPLC). Any other method produces only an estimate of the chlorophyll *a* concentration.

An alternative to the use of physical separation techniques and the distress of choosing the "proper" spectrophotometric equation is to report the amount of total chlorophyll pigments (Golterman and Clymo 1971). It is the estimate of all chlorophyll pigments and degradation products that absorb at 665 nm. The measure is a descendent of the Odum's et al. (1958) monochromatic chlorophyll *a* equation. Golterman and Clymo's equation uses the extinction coefficients of Strickland and Parsons (1963) in 90% acetone which are probably the most popular extinction coefficients and solvent. Their equation is:

$$\text{Total Chlorophyll} = 11.0\,(\text{Abs}_{665} - \text{Abs}_{750})\,\frac{v}{Vp} \qquad (3.1)$$

Where V is the volume filtered (L), v is the volume of extract (ml), and p is the pathlength (cm). Using values for total chlorophyll pigments rather than either the trichromatic equations or the acid-corrected equations gets around the problem of interference by ignoring it. Total chlorophyll is simply a measure of absorbance at 665 nm.

There are some very good reasons for ignoring, or at least giving second place to, chlorophyll *a* values. Comparisons of the total chlorophyll concentration with trichromatic chlorophyll *a* calculated by the Parsons and Strickland (1963) equations using data from the Ohio-NEFCO program have correlations with the trichromatic chlorophylls of greater that 0.99 with a slope of 1. Herve and Heinonen (1982) also reported no significant differences between the Parsons and Strickland (1963) and the "Proposed Norsk Standard" chlorophyll equation, which is identical to that for total chlorophyll pigments. Canfield (personal communication) has not found sufficient amounts of phaeo-derivatives in Florida to warrant reporting anything except total chlorophyll pigments.

In most northern Ohio lakes, correlations between acid-corrected "chlorophyll *a*" and total chlorophyll have a correlation coefficient of 0.96 (n = 88). If, on most occasions, neither interference by other chlorophylls nor by derivatives significantly interfere with the chlorophyll *a* determination, the designation "chlorophyll *a*" could be used. It avoids confusion to use the term "total chlorophyll," which implies no correction for chlorophyll or phaeo-derivatives but also does not perpetuate the myth that chlorophyll *a* can be accurately determined in natural water by spectrophotometric techniques. The major reason to continue to report chlorophyll *a* as well as total chlorophyll would be to accommodate the situations, such as stream periphyton, where considerable degradation products may occur. Carlson generally reports total pigments, chlorophyll *a*, and the acid ratio.

Other Considerations

The bandwidth of the spectrophotometer is important because the wider the bandwidth, the lower the absorbance that is obtained (Weber, et al. 1986). This relationship results from the rather sharp chlorophyll peak. On instruments with large bandwidth, the value includes a greater amount of lower absorption values than would be obtained on an instrument with a narrow bandwidth. Standard Methods recommends using instruments with bandwidth of 0.5 - 2.0 nm.

If phaeo-pigments are to be determined spectrophotometrically, it is necessary to acidify the sample after the first reading. It is important to follow the Standard Methods procedures exactly because any deviation at this step can produce the type of erroneous chlorophyll *a* results mentioned earlier. The amount of acid that has been used in the past has varied considerably. Lorentzen (1968) used several drops of 4M HCl in his original technique, but Riemann (1978) found that such a strong acid causes spectral shifts in the carotenoid, fucoxanthin, which increases in absorbance with acidification and therefore lowers the acid ratio. This shift also increases the value at 750 nm, producing a false increase in the turbidity blank.

A strong acid will also convert phaeophytin, and chlorophyllides to phaeophorbide, which results in an acid ratio greater than 1.7. This results in negative phaeo-pigment values and chlorophyll *a* values higher than total chlorophyll. The amount of time between the addition of the acid and the reading of the absorbance is critical. The conversion from chlorophyll to phaeophytin is a first order reaction, the rate of which is dependent on pH. When strong acids were added, as used in the Lorenzen technique, conversion was instantaneous. The 90 second time recommended in Standard Methods is necessary to complete most of the reaction, yet avoid the interference of degradation products of chlorophyll *b*, which degrades at a much slower rate than those of chlorophyll *a*.

Fluorometric Techniques

When exposed to blue light, chlorophyll molecules will fluoresce brightly in the red region of the spectrum. Fluorometry is a highly sensitive method to determine chlorophyll concentration. This sensitivity can be of value in a volunteer monitoring program because the volunteer will not have to filter as much of a sample than is necessary in spectrophotometric analysis. Even multi-chromatic fluorescence equations exist (Loftus and Carpenter 1971).

Aside from sensitivity, however, there is little to recommend fluorometry over spectrophotometry. There are no independent fluorometric chlorophyll attenuation coefficients, and each fluorometer must be calibrated against spectrophotometric standards. The acid ratio for the acid degradation of pure chlorophyll must also be determined for each instrument.

Weber et al. (1986) also mention problems of the quenching of chlorophyll *a* fluorescence by b-carotene and other accessory pigments, an algal species dependent relationship between extract fluorescence and chlorophyll concentration, and the dependence of chlorophyll fluorescence on temperature. High chlorophyll concentrations will quench the fluorescence, thus requiring the dilution of some samples. Marker et al. (1980) also discourage the use of the fluorometric technique in freshwaters if an acidification step is used to determine phaeo-pigments. Apparently the phaeophytin byproduct of chlorophyll *b* has a fluorescence that overlaps significantly with that of phaeophytin *a*, therefore producing high values for phaeo-pigments. For this last reason, Standard Methods (APHA 1989) does not recommend the acidification step in inland waters when using fluorometry. Total chlorophyll pigments, as discussed above, could be reported.

Methods Used by Programs in the Upper Midwest

Two of the six statewide programs train their volunteers to collect, filter and preserve a water sample for laboratory analysis of chlorophyll *a* concentration, the Indiana Volunteer Lake Monitoring Program and the Wisconsin Self-Help Lake Monitoring Program.

Indiana Volunteer Lake Monitoring Program

Volunteers in the Indiana Volunteer Lake Monitoring Program collect an integrated water sample using a hose that is lowered from the surface to a depth of six feet. The sample is then poured in a pitcher. On shore the volunteer is required to set up a filtering apparatus for the purpose of passing a measured volume of the sample water through filter paper. Algae cells and other suspended matter collect on the filter paper. This paper is then forwarded to the laboratory for chlorophyll *a* analysis.

The actual volume of water to be filtered through the paper is based on the Secchi disk reading. Therefore, the volunteer must be aware of the reading and collect enough sample water to satisfy the filtration volume requirements for the chlorophyll *a* analysis. Table 3.2 presents the range of Secchi disk depths and corresponding volumes of water to be filtered. Listed below are the instructions for filtering the chlorophyll *a* sample generalized from a training document given to Indiana volunteers.

- Set up the work area out of direct sunlight.
- Separate the chlorophyll *a* filtration apparatus by unscrewing the upper chamber from the receiver.
- Pick up one of the 4.7 cm filters with tweezers and place the filter on the filter support plate on top of the receiver.
- Carefully place the upper chamber back on top of the filter support and receiving flask.
- While holding the upper chamber piece stationary, tighten the locking ring until the upper chamber is firmly seated on the receiver. (Do not overtighten the locking ring or allow the upper chamber to rotate while tightening because this may tear the filter paper.)
- Connect the tubing from the hand pump to one of the two side-arms on the side of the receiver. (The other side-arm must have a black cap on it in order for a vacuum to form.)
- Determine the amount of sample water to be filtered from the chart. Pour the correct amount from the pitcher into the 500 ml graduated cylinder. If more than 500 ml is needed, the volunteer will need to refill the cylinder after passing the initial 500 ml.
- Pour water from the graduated cylinder into the upper chamber. Squeeze the vacuum pump until the pressure on the dial reaches 15 psi. Do not exceed 15 psi or the pressure or you may damage the filter. Over time the pressure will decrease so you will need to periodically squeeze the hand pump to maintain pressure.
- The upper chamber and receiver only holds 500 ml each. If you are required to filter more than 500 ml you must disassemble the apparatus and empty out the receiver.
- When you are through filtering the recommended amount of lake water, remove the upper chamber.
- Using the tweezers, carefully pick up the edge of the filter and fold it in half on top of the filter support plate. (All of the algae and other particles are trapped on top of the filter paper, therefore this "green" top side of the filter must always be on the inside of the fold.)
- Using the tweezers, pick up the edge of the filter paper and fold it in half again.
- Place the folded filter paper into the opaque amber chlorophyll sample bottle and label it with the lake name, date and number of milliliters of sample water filtered.
- Place the bottle in the styrofoam mailer and put the mailer in the freezer. Samples must be frozen immediately.

The volunteer is instructed to contact program officials once two samples have been collected. Officials then arrange with the volunteer a convenient time for pickup by a shipping company.

Wisconsin Self-Help Lake Monitoring Program

Volunteers in the Wisconsin Self-Help Lake Monitoring Program take a point sample three feet below the surface for chlorophyll *a* analysis. Like their counterparts in Indiana, Wisconsin volunteers also assemble a filter apparatus and filter a measured volume of sample water through filter paper. The actual volume to be filtered is based on the Secchi disk reading and determined using the same chart used in Indiana's program (see Table 3.1).

Listed below are the instructions for filtering the chlorophyll *a* sample excerpted from the training manual given to Wisconsin volunteers. (Excerpts have been slightly edited by the authors to enhance the continuity of this report.)

- Set up a work area in the shade, out of direct sunlight.
- Assemble the filtering apparatus and attach the plastic tubing of the hand pump to the flask.
- Pick up one small filter paper with the tweezers and place it on the center of the filter base.

Squirt a small amount of distilled water on the paper to keep it in place.

- Carefully place the cup on top of the filter base. It is magnetic. Be sure that the filter paper does not move!
- To determine the volume of water to be filtered, refer to the chart that matches Secchi disk depth with volume of water to filter.
- Take out the plastic jug filled with the water for the chlorophyll sample. Gently mix the water in the jug by turning it upside down several times. Fill the graduated cylinder to the appropriate level. Since the graduated cylinder holds only 500 mls, you may need to fill it more than once. If so, keep track of the total volume of water filtered.
- To begin filtering, pour some of the measured water from the graduated cylinder into the top cup of the filter apparatus. Squeeze the hand pump until the water has moved through the filter. (Note: The bottom plastic flask only holds 1,000 mls, so if you must filter more than 1,000 mls, the flask will have to be emptied. Be careful to not disturb the filter paper.)
- If you have not filtered all the water after 20 minutes, you may stop. Be sure to record the volume of water you did filter, not what you were supposed to have filtered. Do this by looking at the graduated cylinder and determining how much you have used.
- When you are finished filtering, separate the top cup from the filter base. Using the tweezers, fold the filter paper in half so that the algae is on the inside. Fold it in half again and wrap it in a small piece of aluminum foil.
- Using a waterproof Sharpie pen, complete the information on a chlorophyll sticker. Attach the sticker directly to the aluminum foil packet.

The volunteer is then instructed to place the bottle next to the bottle of ice and mail it as soon as possible to the State Lab.

A test of the quality of volunteer technique was made in 1990 (Betz et al., 1990). Fifteen lakes were visited by a Wisconsin DNR employee. The employee both observed the volunteer and returned duplicate samples immediately to the laboratory for analysis. Several problems were observed by the DNR personnel. The glass fiber used was slightly smaller than the outside diameter of the filter holder and, if care was not used, the filter would not be sealed, and leaks would occur when the apparatus was disassembled. Pressure created inside the filter flask when the assembly was reassembled would cause the filter to buckle.

Another problem encountered was that some volunteers were spending too much time filtering because they filtered too much water (1500 ml). The problem was addressed by having the volunteers change the filter paper after the rate of filtering declined. All the filters were combined in the subsequent analysis. Betz (1992) reported that Secchi disk transparency is now used as an indicator of the amount of water to filter (see Table 3.1).

In 1991, a comparison of volunteer versus WDNR personnel chlorophyll values had a r^2 of 0.83 (Glaser 1992). Duplicate samples taken by the volunteer had an r^2 of 0.98. Although the agreement between volunteer and personnel was good, Glaser thought that there was too much variation in length of storage. He recommended that samples be shipped inside ice packs.

Other Programs

Florida

Officials in the Florida Lakewatch program supply volunteers with a complete filtration system; filters (Gelman Type A glass fiber filters), hand pump, plastic filter funnel and base (Gelman), filtration flask, etc. The sample is collected in a gallon jug and returned to the volunteer's home for filtration. The jug is shaken gently to mix the sample and the sample poured into a graduated cylinder. The volunteer is instructed to use a volume (ml) of approximately 100 times the Secchi disk reading (in feet). More water is added if the filter is not slightly green. After filtration, the filter is removed, folded, and placed inside a large, labeled, folded, filter paper. This large filter paper serves to protect the chlorophyll filter. The filters are then placed in a jar of silica gel and frozen. They are taken to a collection center within three months.

Florida has made a comparison between measurements made by the volunteers and made by professional working on the same lakes. The nature of the sampler (volunteer or professional) accounted for less than 1% of the variation in the relationship. More important sources of variation were lake to lake differences (69%) and seasonal differences (29%), and station differences (<1%) (Canfield 1991).

Minnesota

The Minnesota Clean Water Partnership program recommends taking the sample in pre-cleaned polyethylene bottles. The samples are filtered until the filter turns a light green color. Filters are placed in opaque containers or petri dishes wrapped in foil and stored on dry ice. Samples are analyzed as soon as possible after collection according to Standard Methods (APHA 1985). Both chlorophyll *a* and phaeophytin *a* are reported (MPCA 1989).

New York

Volunteers in the Citizens Statewide Lake Assessment Program are supplied with a complete filtration

system; filters, hand pump, plastic filter funnel and base (Gelman), filtration flask, etc. The sample is collected in a Kemmerer bottle and poured into a collapsible container. Once on shore, a "few drops" of $MgCO_3$ are added to the filter and 10-25 ml of sample are filtered. After filtration, the filter is removed, rolled into a cylindrical tube, and placed inside a boro-silicate vial. The tube is capped, labeled, and wrapped in aluminum foil. The tubes are placed in mailing boxes with two ice packs and mailed to the laboratory within 6 hours after collection and filtration.

Ohio (NEFCO)

This program uses the in-line Swinney filter system described above. The volunteer filters an amount of water based on the Secchi disk transparency (see Table 3.1). The filter and filter holder are then wrapped in aluminum foil, labeled, and frozen. A NEFCO employee picks up the samples within a few days and brings them to the laboratory. The samples remain frozen until analyzed. Analysis is according to Standard Methods (APHA 1989).

Recommendations

Sampling, Filtration, and Preservation

Samples should be filtered as quickly as possible. In the interim, the water samples should be kept cool and dark. The type of filtration apparatus can be left to the discretion of the coordinator. Providing a complete filtration apparatus has the single advantage of allowing the volunteer to see the color of the filter and thus judge the amount of algae to filter. Its disadvantages are that it is expensive and it requires considerable amount of manipulation and care by the volunteer.

The advantages of using the Swinney filter holder are that it is relatively inexpensive, requires no manipulation by the volunteer, and the filtration is done immediately, therefore requiring no sample bottle or sample preservation. Its disadvantage is that the volunteer does not open the holder, and therefore cannot judge if sufficient sample has been filtered. If the volunteer does not dry the filter, there is a greater chance that the filter holder will contain residual water that might promote growth or degradation of the chlorophyll.

Keeping the chlorophyll molecule intact until analysis is a concern. Most programs immediately freeze the sample. Freezing seems to provide adequate preservation for at least several months. Some programs also keep the filters dry by adding silica gel to the containers holding the filters. Although mailing of the frozen sample to the laboratory is done, experience suggests that unless it can be ensured that the sample is delivered within 48 hours, there is a possibility of degradation. Immersing the filter in the solvent or drying the filter are possible alternatives that should be given consideration. The safest method is to have the samples picked up by program personnel.

Analysis

A number of combinations and variations of the chlorophyll technique exist. The important fact is that the final chlorophyll value is highly dependent on the technique used. Without standardization, program to program comparisons of chlorophyll values should be held as suspect.

The concept behind the manual, *Standard Methods*, is to provide just that, a standard set of methods for all analysts. The chlorophyll method described in APHA (1989) is recommended. This technique, since it uses acetone as the solvent, probably does not provide total extraction of chlorophyll from some algal cells, but does provide some analytical consistency with historical data.

Using a different extractant will undoubtedly change the amount of chlorophyll extracted from the filtered cells. It will therefore change any empirical relationships between chlorophyll and other limnological variables such as phosphorus, Secchi depth, etc. Changing extraction solvents should be done only with the knowledge that published empirical relationships may no longer be valid.

The sole use trichromatic equations are not recommended: they take longer and can provide erroneous chlorophyll *a* values. At best, the chlorophyll *a* values are equal to those for total chlorophyll pigments.

Chlorophyll *a* values obtained after acidification can be reported but remember that calling the phaeopigment corrected value "chlorophyll *a*" does not make it so. It would be better termed "magnesium-containing pigments." This value is dependent on the technique and would be expected to vary widely from procedure to procedure.

It is strongly recommended that the total chlorophyll pigment be reported in addition to chlorophyll *a*. This value, although flawed by interferences by other chlorophylls, phaeo-pigments, as well as a number of other possible interferences, is the only value that remains fairly independent of chlorophyll methodology. Therefore, it is the only measurement that provides historical consistency.

Chlorophyll *a* methodologies have changed over the past 25 years, and with each change, the previous chlorophyll estimates became obsolete and non-comparable to the new methods. If everyone had reported total chlorophyll, at least there would be one consistent value that would allow comparison. In a monitoring program, where historical data consistency is absolutely necessary, this value should be reported.

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4. Phosphorus

Phosphorus is probably the most studied plant nutrient in freshwater aquatic sciences. It is often found to be (and more often inferred as) the nutrient that limits the growth and biomass of algae in lakes and reservoirs. Whether this nutrient is as universally limiting as once believed is debatable, but certainly there is substantial evidence of its importance in many lakes. Numerous correlations and regressions have been constructed linking phosphorus, especially total phosphorus, with variables such as algal chlorophyll, algal weight, and productivity. Because of its possible importance in limiting the growth and biomass of algae and because of the numerous empirical models available for phosphorus, it is an important addition to the list of variables to be measured in volunteer programs.

Forms of Phosphorus

Phosphorus in natural waters is divided into three component parts: soluble reactive phosphorus (SRP), soluble unreactive or soluble organic phosphorus (SUP) and particulate phosphorus (PP) (Rigler 1973). The sum of SRP and SUP is called soluble phosphorus (SP), and the sum of all phosphorus components is termed total phosphorus (TP). Soluble and particulate phosphorus are differentiated by whether or not they pass through a 0.45 micron membrane filter. The phosphorus fractions are illustrated in Fig. 4.1.

Soluble Reactive Phosphorus (SRP)

This phosphorus fraction should consist largely of the inorganic orthophosphate (PO_4) form of phosphorus. Orthophosphate is the phosphorus form that is directly taken up by algae, and the concentration of this fraction constitutes an index of the amount of phosphorus immediately available for algal growth.

In phosphorus limited situations, the concentration of this form should be very low to undetectable (<5 µg/L). As concentrations of orthophosphate (as reflected in the SRP fraction) increase, it can be inferred that phosphorus is either not needed by the algae or that it is being supplied at rates faster than it can be taken up by the biota. Measurement of SRP can be used as an indicator, albeit a potentially inaccurate one, of the degree of phosphorus limitation of the algae.

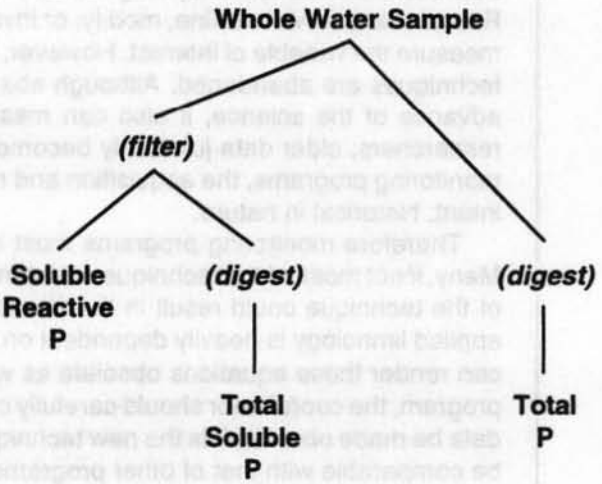

Figure 4.1—The phosphorus fractions found in lake water.

At one time SRP was called "dissolved inorganic phosphorus." This terminology was changed to "soluble reactive phosphorus" (Rigler 1964; Strickland and Parsons 1965) to reflect a more realistic interpretation of what forms of phosphorus were found in this fraction. The terms "soluble" and "reactive" were chosen instead because this form of filtered phosphorus was neither necessarily dissolved nor necessarily inorganic.

The term "reactive" is used to indicate that the phosphorus in the SRP fraction is not solely inorganic phosphorus, but could include any form of phosphorus, including some organic forms, that react with the reagents. Some organic forms apparently do hydrolyze and react under the conditions of this test, while some forms of inorganic phosphorus (polyphosphates), in fact, do not react. There is a continuing debate as to what extent SRP represents solely the ortho- form of phosphorus or is biologically available (Nürnberg and Peters 1984). The actual composition of SRP probably varies with the nature of the water body.

The "soluble" fraction does not necessarily contain only dissolved phosphorus forms: the phosphorus-containing material in the soluble fraction is dependent on the porosity and characteristics of the filter used. Typically, a 0.45 µ cellulose (Millipore) filter is used as a standard. This filter excludes most particulates, but colloidal phosphorus may be present in the filtered fraction.

Some protocols use glass fiber filters instead of a membrane filter. Using a glass filter increases the amount of particulate material that passes through the filter and therefore increases the amount in the "soluble" fraction. Small particulates, including very small algae and bacteria will be present in the filtered sample. Whether or not they become represented as SRP will depend on the extent that they react with the reagents. Glass fiber filters are less costly than the membrane filters, and, more importantly, are used in a number of other tests such as chlorophyll, particulate carbon, and suspended solids. There is no consensus as to whether the necessity of separating the soluble fraction from all possible particulate forms is more important than analytical consistency between variables.

Some researchers in phosphorus dynamics use Nucleopore filters instead of either membrane or glass fiber filters. These filters partition the water into size fractions more accurately than do membrane or glass fiber filters, and therefore allow much better separation of size-related phosphorus forms. Nucleopore filters also do not contain phosphorus, which is a contaminant in membrane filters (Peters, personal communication). However, these filters are more expensive than either membrane or the glass fiber filters, and the increased control of size may not be important to a general monitoring effort.

Soluble Unreactive Phosphorus (SUP)

This phosphorus fraction contains filterable phosphorus forms that do not react with the phosphorus reagents under the time and conditions of the test. It is measured as the difference between soluble phosphorus (SP) and SRP. The compounds in the SUP fraction

A Continuing Debate

The debate over choice of membranes reflects a continuing, and generally healthy, conflict between the need for advancing research techniques and the needs for historical consistency. Researchers strive to refine, modify, or invent new techniques that more accurately or precisely measure the variable of interest. However, with each advance of technique, older, less accurate techniques are abandoned. Although abandoning less accurate techniques is healthy for the advance of the science, it also can mean the discarding of older, less accurate, data. For researchers, older data justifiably become obsolete with each new technical advance, but for monitoring programs, the acquisition and retention of monitoring and survey data is, by its very intent, historical in nature.

Therefore monitoring programs must be conservative in the adoption of new techniques. Many, if not most, of the techniques of interest in this manual are operationally defined. A change of the technique could result in the abandonment of years of previously collected data. Also, applied limnology is heavily dependent on empirical predictive equations; changing techniques can render these equations obsolete as well. Before choosing or changing techniques for the program, the coordinator should carefully consider the impact of the choice. Will older, important data be made obsolete? Is the new technique generally recognized and accepted? Will the data be comparable with that of other programs? Is the increased accuracy or efficiency necessary to the goals of the program?

are organic forms of phosphorus and chains of inorganic phosphorus molecules termed polyphosphates. The size of this fraction relative to the other phosphorus fractions is highly dependent on the type of filter used to separate the soluble from particulate fractions.

A number of organic phosphorus molecules have been identified, but two main classes seem to predominate in natural waters. The first is low molecular weight compounds, apparently derived from algal and bacterial metabolism, which release orthophosphate upon treatment with alkaline phosphatase. These compounds do not react with the phosphorus reagents without prior digestion (Franko and Heath 1979). The second is colored, large molecular weight compounds, perhaps phosphorus bound in humic complexes, which release orthophosphate in the presence of ultraviolet light.

These compounds may (Downes and Paerl 1978) or may not (Franko and Heath 1979) react without prior digestion and could be measured as SRP. These organic forms apparently form a pool of phosphorus for algal and bacterial growth, but they must be first converted to orthophosphate, either by enzymes or by UV light, before they are available for uptake by the biota. Although the phosphorus in the pool appears to be highly dynamic, the total amount of phosphorus in this fraction seems to be fairly stable seasonally in lakes with long residence times (Rigler 1964). However, changes in the pool might signal shifts in either the availability of this phosphorus form to algae or bacteria or shifts in the biotic community's ability to use this form, or more likely, runoff variability into the lake.

Soluble Phosphorus (SP)

This form is measured after the digestion of the filtrate and should contain all filterable forms of phosphorus, both organic and inorganic that are converted to orthophosphate by the digestion process. However, the amount of phosphorus in this filterable pool is highly dependent on the filter used. The larger the effective pore size of the filter, the more particulate material that will pass through the filter, be digested, and be considered "soluble."

Rigler (1964) estimated the percent of phosphorus that would be considered part of the soluble phosphorus pool of three lakes, using 7 different separation techniques and filter sizes. Particulates were removed using a Foerst centrifuge, or by passing the water through 3 layers of Whatman #44 paper filters, or through 5.0, 1.2, 0.45, 0.22, and 0.1 micron Millipore filters. Considerable differences in the percent soluble phosphorus were found as the pore size decreased (Figure 4.2).

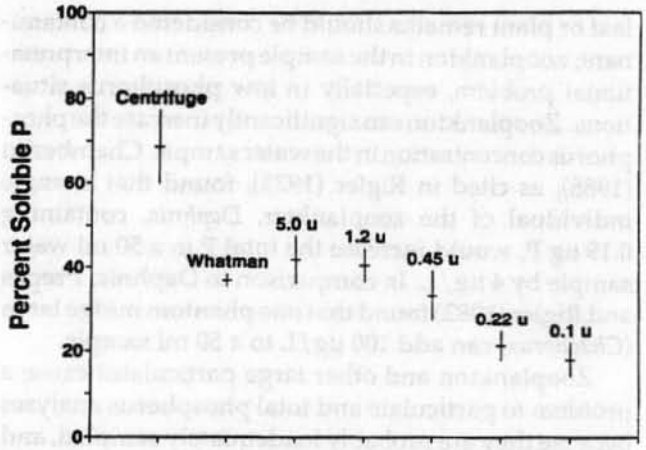

Figure 4.2—The effect of particle separation method or filter pore size on the estimated amount of soluble phosphorus in lakewater (mean ± SE). Data from Rigler 1964.

Deviation from the 0.45 μ membrane standard will have a significant affect on the soluble phosphorus concentration, and, because it is calculated as the difference between SP and SRP, soluble organic phosphorus will be affected as well. SRP may or may not be affected, depending on the extent to which phosphorus in these particulates are released during the analytical procedure.

Particulate Phosphorus

This fraction of phosphorus contains all material, inorganic and organic, particulate and colloidal, that was captured on the filter. Typically, particulate forms will contain bacteria, algae, detritus, and inorganic particulates such as clays, smaller zooplankton, and occasionally, larger zooplankton, sediments, or large plant material. Particulate phosphorus can be measured either by filtering a known volume of water through a membrane filter and then digesting the filter, or it can be obtained by subtraction of SUP from the total phosphorus concentration. The filtration method allows the analyst to concentrate samples from low-particulate waters, thereby increasing the sensitivity of the test, but it also increases the probability that large particles, such as zooplankton will be captured as well. Caution must be taken so that adequate oxidant is present to completely digest the sample.

Large zooplankton, suspended sediments, or large plant remains may be captured on the filter. These latter particulates are generally considered to be contaminants rather than normally occurring portions of the fraction. Although suspended sediments, especially if stirred up during the sampling process or stray

leaf or plant remains should be considered a contaminant, zooplankton in the sample present an interpretational problem, especially in low phosphorus situations. Zooplankton can significantly increase the phosphorus concentration in the water sample. Chamberlin (1968), as cited in Rigler (1973), found that a single individual of the zooplankter, *Daphnia*, containing 0.19 µg P, would increase the total P in a 50 ml water sample by 4 µg/L. In comparison to Daphnia, Prepas and Rigler (1982) found that one phantom midge larva (*Chaoborus*) can add 100 µg/L to a 50 ml sample.

Zooplankton and other large particulates cause a problem to particulate and total phosphorus analyses because they are probably inadequately sampled, and therefore give an inaccurate phosphorus estimate. If particulate P is determined by subtraction, zooplankton are probably not sampled accurately or randomly in a 10 or 50 ml aliquot used in the total phosphorus determination. The presence or absence of the few zooplankton that may be captured would cause a significant overestimate or underestimate of the limnetic phosphorus pool if zooplankton are considered part of that pool. Although it might seem that filtering and digesting the filter would increase the precision of the particulate measurement, its accuracy is still in doubt because the tools used for collecting total phosphorus from the lake (water samplers or tube samplers) will generally undersample zooplankton populations. By either method, the limnetic phosphorus pool will be inaccurately measured.

The larger size fraction of the limnetic phosphorus pool (zooplankton and fish) is generally ignored in most studies, although they may make up a sizeable fraction of the total phosphorus in the water. For example, Prepas and Rigler (1982) found that the phosphorus fraction greater than 250 microns (which might contain larger algae as well as zooplankton) constituted between 14 and 28% of the total phosphorus in the epilimnion on an Ontario lake. Fish can also constitute a sizable proportion of the total phosphorus in a lake. Kitchell et al. (1975) reported that zooplankton constituted 18% and fish, 74%, of the total pelagic zone phosphorus in Lake Wingra, Wisconsin. Generally these larger size fractions do not interfere with the use of total phosphorus in empirical models because the data used in model construction probably ignored these size fractions as well. However, if the data were used to estimate lake phosphorus content for a nutrient budget, then it is possible that the content could be seriously underestimated without consideration of the larger fractions.

The ideal would be to have a size-fractionated phosphorus, where phosphorus would be sampled by methods appropriate for each size fraction. In the absence of the ideal, which is probably the case of volunteer programs, particulate phosphorus is probably best measured by subtraction from total phosphorus rather than filtration. In this case, the particulate value will be influenced by the pore size of the filter used. Volunteers could be cautioned to try to exclude large particles such as leaves.

Total Phosphorus

This form incorporates the total of all filterable and particulate phosphorus forms mentioned above. It is probably the most-often analyzed fraction of phosphorus because it is:

- used in a wide variety of empirical models relating phosphorus to a wide variety of limnological variables (Peters 1986); and
- the link between phosphorus loading estimates and phosphorus content in the lake.

Considering the wide variety of materials that might constitute "total phosphorus," it is remarkable that total phosphorus correlates well with any other single variable, especially an algal variable such as chlorophyll. The chlorophyll molecule itself does not even contain phosphorus. For such correlation to be strong, chlorophyll must be related to phosphorus- containing compounds in algal cells. Variations in TP-chlorophyll relationships come, in part, from variations in the amount of chlorophyll generated per unit of total phosphorus in algal cells. As cells vary in their phosphorus or chlorophyll content, the TP/chlorophyll ratio will vary. All other phosphorus forms found in the water must be constant, negligible, or at least change as a function of chlorophyll.

For example, SRP is generally low in phosphorus limited situations while SUP may be relatively constant. Increases in SRP or variations in SUP would adversely affect chlorophyll-phosphorus relationships. Forms of particulate phosphorus other than algal phosphorus, must also be negligible, constant, or change as a function of chlorophyll. Chlorophyll would be expected to relate best to total phosphorus in situations where there are negligible amounts of clays, suspended sediments, and detritus, where phosphorus limits algal biomass, and in deep natural lakes so that bottom sediments are not resuspended.

"Total" phosphorus is largely defined on the basis of how much phosphorus in its various forms will be oxidized into orthophosphate by a specific oxidant. Different analytical tests used for the digestion and analysis of phosphorus may change the amount of phosphorus reported. It is important to remember that all these phosphorus designations are functionally defined, and the coordinator must be very cautious about modifying phosphorus analysis procedures. It is possible that some modifications would alter the results significantly and therefore limit the use of the

data. It is also important to remember that all of the empirical equations are based on certain methodologies (hopefully, the same ones). Deviations from these methods could introduce error in these empirical relationships (Griesbach and Peters 1991).

Analysis

Although a number of analytical tests exist for the measurement of phosphorus, the ascorbic acid method described in Standard Methods (AHPA 1989) is probably the most commonly used test. In this test, the molybdate reagent reacts with orthophosphate, producing phospho-molybdic acid, which forms the colored molybdenum blue upon reduction with ascorbic acid. While the compound appears blue, the peak absorbance at 885 nm is in the infra-red region. Absorbance is linearly related to concentrations by Beer's Law, and this test detects phosphate concentrations of 5 to 1300 ug/L with a cuvette pathlength of 1 cm.

Lower limits of detection can be obtained with a longer pathlength cell. Griesbach and Peters (1991) found that TP concentrations (after digestion) were relatively insensitive to even substantial changes in the concentration of the phosphorus reagents, and high precision was unnecessary when preparing the reagents. Results should be reported as the concentration of phosphorus, not phosphate, present in the water. Since orthophosphate is the form measured in the test, the results can be presented as PO_4-P.

As mentioned earlier, total phosphorus and total soluble phosphorus require a digestion step prior to the measurement of the ortho-phosphate form. There is no one perfect oxidant. The acid-persulfate test that is often used is a compromise method that extracts most but not all phosphorus, but is a safer technique than others. Perchloric acid digestion, for example, will extract phosphorus from soils, but is a more complicated procedure and requires special facilities and safety precautions. Harwood et al. (1969) compared three different digestion methods (magnesium nitrate fusion, persulfate digestion, and peroxide digestion) on five types of liquid samples but found no significant differences in the phosphorus concentrations.

Prepas and Rigler (1982) found that using persulfate alone produced less variable results than did perchloric acid or potassium persulfate with sulfuric acid (Jeffries et al. 1979). Standard Methods (1989) recommends the use of acid together with persulfate. It may be that the acid aids in the digestion of phosphorus from clays and other soil particles. Griesbach and Peters (1991), in an extensive examination of phosphorus techniques, found that the total phosphorus values resulting from varying the amount of persulfate added was not important within a range of 0.5 to 0.8 g persulfate per 40 ml sample. They suggested that weighing of each sample was not necessary; using a simple, volumetric scoop of persulfate was adequate. They also found that digestion time and whether the samples were autoclaved or boiled had no significant effect on the final phosphorus concentration. They suggest that boiling is more time-consuming than autoclaving but can be used if no autoclave is available.

Several substances can interfere with the phosphorus analysis. Arsenate, which has been used to control algae and aquatic macrophytes, and high concentrations of silica can interfere. Where there is considerable humic color or clay turbidity, it may be necessary to run color/turbidity blanks for each sample. Keep a close watch on the physical characteristics of water samples. The phosphorus test is sensitive to trace amounts of contamination as is normally present in tap water, on fingers, in soap and some detergents, and in buffers or other reagents. It is important, therefore, to clean glassware thoroughly, and observe maximum cleanliness in the field collection and in the laboratory. It is also very important to run distilled water blanks and standards, keeping records of the absorbance to detect changes in distilled water quality or reagents.

It is also important to have an appropriately defined TP detection limit. For example, a TP detection limit of 50 µg/L will not be adequate for a great deal of limnological efforts. As a matter of protocol, detection limits of 10 or 5 µg/L (or lower) will likely be necessary, to have adequate resolution for typical stream and lake monitoring. Using a rule of thumb that the detection level is about two times the standard deviation of replicate blanks, the lowest level of quantification is about five times this standard deviation. Hence, the level of quantification better defines the lower levels of identifiable TP concentrations, which occur with better (lower) detection levels.

Methods Used by Programs in the Upper Midwest

The Illinois, Indiana, Michigan and Wisconsin Programs train their volunteers to collect a water sample for the analysis of total phosphorus.

Illinois Volunteer Lake Monitoring Program

Volunteers in the Illinois Program collect a point water sample at one foot depth for an analysis of several parameters including total phosphorus. The sample is poured into a 4 oz. bottle that also contains a small amount of nitric acid. The acid preserves the sample until it can be analyzed. The sample is refrigerated and shipped to the Illinois EPA laboratory as soon as possible after collection.

Indiana Volunteer Lake Monitoring Program

Volunteers in the Indiana Program collect an integrated water sample using a hose that is lowered from the surface to a depth of six feet. The sample is then poured in a pitcher container. The volunteer is also provided a 125 milliliter clear bottle that has been acid-washed. This bottle will contain the sample that will be analyzed in the laboratory for total phosphorus concentration.

Listed below are the instructions for filling the phosphorus sample bottle generalized from the training document given to Indiana volunteers.

- Swirl the pitcher to thoroughly mix the water.
- Carefully pour the water from the pitcher into the phosphorus bottle. Be careful to not let the mouth of the bottle touch the pitcher or anything else.
- Fill the bottle up to the bottom of the neck in order to allow for expansion of the water when you freeze it.
- Secure the cap onto the bottle.
- Label the total phosphorus bottle with the lake name and date sampled.
- Place the bottle in the styrofoam mailer and put the mailer in your freezer. Samples must be frozen immediately.

The volunteer is instructed to contact program officials once two samples have been collected. Officials then arrange with the volunteer a convenient time for pickup by a shipping company.

Michigan Self-Help Lake Monitoring Program

Volunteers in the Michigan Program collect a total phosphorus sample and duplicate one foot below the water surface approximately two weeks after ice leaves the lake in the spring. At the time of sampling the volunteer uses the specific sampling instructions to secure the water samples. The volunteer then labels the bottles with their name, the name of the lake, county and the date the sample was collected. The samples are immediately frozen and within three days delivered to a MDNR field office. All samples are then next day mailed in a shipping cooler to MDNR's headquarters in Lansing. Samples arrive in Lansing still in a frozen state. The samples are allowed to thaw, then acid preserved and delivered to the MDNR laboratory for analysis.

For 1994, the MDNR is planning side-by-side sampling with 10 to 15 percent of program participants. This program element is being implemented to increase program interest, provide additional training, allow on-site assessment, and evaluate data collection.

Wisconsin Self-Help Lake Monitoring Program

Volunteers in the Wisconsin Program take two point samples for phosphorus analysis. Using a horizontal point sampler, one sample is collected from a depth of three feet. The second sample is collected three feet off the bottom. Each sample is poured into its own 250 ml bottle and labeled. Sulfuric acid is then added to preserve the sample.

Listed below are the instructions for preparing the phosphorus sample for shipment to the state Laboratory of Hygiene generalized from the training manual given to Wisconsin volunteers.

- Put on safety gloves and glasses.
- Take out the first (three-foot depth) phosphorus sample that you collected.
- Remove a sulfuric acid glass ampule from the square plastic container. Gently tap the neck portion of the ampule with your fingers to move any residual acid down into the base of the ampule.
- Insert the glass ampule into the plastic sleeve.
- Snap open the ampule by firmly but gently grasping either side of the ampule neck with your thumb and forefinger. Press the etched area on the ampule neck with your thumb while holding on to the base of the ampule with your other hand.
- Turn the glass ampule upside down and pour all the acid into the plastic collection bottle marked 3 foot by tapping the bottom of the ampule with your index finger.
- Place a yellow "Sulfuric Acid Added" sticker on the plastic phosphorus bottle.
- Store the broken ampule pieces in the plastic waste bottle provided with the State Hygiene Lab field kit. You may sprinkle a little baking soda into the bottle to neutralize any residual acid.
- Repeat the steps for the second phosphorus sample collected three feet off the bottom.
- Complete a data sheet for each of the two preserved phosphorus samples.
- Store the preserved phosphorus samples in the refrigerator until you are ready to mail them. Do not freeze these samples!

The training manual emphasizes that the sulfuric acid must be handled with care and according to the directions. Safety instructions are also provided about what to do if acid accidentally spills or comes into contact with the eyes or skin. The samples are then packed in a mailer box with the data sheets, chlorophyll filter, and an extra plastic bottle containing ice. The volunteer is instructed to mail the samples to the state laboratory as soon as possible after collection.

The field QA/QC program consisted of duplicate samplings by the volunteer and a program field technician. The technician performed the same field and laboratory sampling techniques, and mailed in the samples in separate mailers. Volunteers collected duplicate phosphorus samples without using the sampler. Instead they held one of the nutrient sample bottles at the one foot depth (sample depth has since been changed to three feet) and manually removed the cap, letting the bottle fill, and then, replacing the cap under water. Each person also submited a "blank" sample by rinsing the sampler with distilled water, and then passing more distilled water through the sampler and into the sample bottles (Betz et al., 1990).

These authors reported that several people had difficulty emptying the sulfuric acid ampules into the sample, and, although the volunteers have been given safety goggles and safety gloves, very few people used either of these. They also reported that volunteers found it difficult to collect the phosphorus sample two feet off the lake bottom. Wisconsin has since changed to bottom sample to three feet.

Other Volunteer Programs

Florida

Volunteers in the Florida Lakewatch program ask the volunteers to take three bottles with them in the boat: a clean, empty gallon jug with a lid (for chlorophyll analysis), and two small plastic containers with lids, provided by the Program, for nitrogen and phosphorus analysis. The small plastic containers have been pre-cleaned by the University. The sampling procedure follows the acronym, "RIP UP," which means, "Rinse, Invert, Plunge, Up-end, and Pour." Samples are taken at elbow depth. Some water is poured from the small containers until there is a one-inch air space. The containers are subsequently labeled, put in a "baggie," frozen, and stored in the volunteer's freezer for up to 3 months before being taken to the Program Collection Center.

Florida has made a comparison between measurements made by the volunteers and made by professionals working on the same lakes. The nature of the sampler (volunteer or professional) accounted for less than 1% of the variation in the relationship. More important sources of variation were lake to lake differences (82%), seasonal differences (17%), and station differences (<1%) (Canfield 1991).

Ohio (NEFCO)

This Program has a smaller, second tier program that measures total phosphorus and chlorophyll. The volunteer takes a sample from the 3 foot depth with a commercial water sampler. A 50 ml sample is withdrawn from the sampler using the syringe apparatus described in the sampler section. The sample is injected into a pre-cleaned borosilicate bottle cleaned and provided by the Program. The volunteer labels the bottle and then puts the bottle into the refrigerator until picked up by a Program employee.

New York

The New York program collects a sample with a plastic Kemmerer bottle. The water is transferred into a collapsible container. On shore, the 100 ml of the water is transferred into a marked container for later total phosphorus analysis. These containers contain preservatives (1 ml 5N sulfuric acid), and the volunteers are warned to wear vinyl gloves when dealing with the sample. Later, the samples are placed with other same containers and chlorophyll *a* tubes in a styrofoam shipping container, and shipped together with two ice packs to the laboratory for analysis.

Recommendations

Sampling and Preservation

Although field kits that measure phosphorus potentially could be used by volunteers, the limits of detection of the kits that use visual comparators are too high to be useful in lake monitoring programs. A sensitive spectrophotometer is necessary to measure the phosphorus levels found in most lakes. Because of this, volunteers will most often be asked to collect samples for later analysis in a laboratory. Prime concerns should be that the volunteer collects the sample correctly and that the sample is stored in a manner that will allow subsequent ānalysis with minimal changes in either the absolute concentration or phosphorus form.

Collection of the water sample for subsequent analysis can be done with any of the sampling techniques discussed in the chapter on sampling techniques. Surface (0.5 m) samples are usually used in trophic state determinations, but see the chapter on sampling for a discussion of alternative sampling procedures. Whatever the technique chosen, the phosphorus sample should be taken from the same water sample that is used for chlorophyll. Hypolimnetic phosphorus samples can also be taken, but as the Wisconsin program discovered, it is difficult to obtain a near- sediment sample without disturbing the sediments. Care should be taken that the sampler is clean, without any dirt or material from earlier excursions clinging to the inside surface. Several rinsings with lake water prior to use may be beneficial.

Once collected, the water should be poured into a pre-cleaned sample container. It would be best that this sample container be cleaned and supplied by the program rather than relying on the volunteer to sup-

ply or to clean the container. Cleaning should consist of washing the containers in phosphorus-free detergent, several tap water rinses, a rinse in dilute HCl (Standard Methods APHA 1989, recommends hot dilute HCl) and then several rinses in distilled water. If care is not taken in the cleaning of the containers, subsequent care becomes meaningless. Standard Methods (APHA 1989) also recommends that plastic jars not be used unless the sample is frozen because the phosphorus may be adsorbed onto the walls of the plastic container. If the sample is to be poured from the container in the laboratory prior to analysis, the sample should be preserved prior to storage to prevent any transformations (uptake by algae or bacteria) or sorption to the walls of the container during storage. If only total phosphorus will be measured, then the sample can be preserved with one ml of HCl per liter or by freezing the sample.

An alternative method used in several programs is to measure and pour an exact amount of the sample into a pre-cleaned borosilicate bottle. Later, digestion is done in this same container. The assumption is that preservation is not necessary because any material adsorbed onto the side of the container will be stripped off during the digestion process.

Griesbach and Peters (1991) found that TP in unpreserved, unfiltered samples stored frozen was stable for up to 12 months, if stored in the tubes in which they would eventually be analyzed. At room temperature, samples could safely be stored for a month, again, if stored in the tubes in which they would be analyzed. If other forms are to be analyzed, filtration prior to preservation seems to yield the best results. If clean filtration equipment can be ensured, there is no reason that the volunteers could not do the filtration to differentiate between soluble and particulate phosphorus. If glass fiber filters were used, then the filtrate obtained from the chlorophyll filtration could be used for the soluble phosphorus fraction. The problem of volunteer- obtained filtered samples is that of preservation. Standard Methods (AHPA 1989) recommends that the sample should be immediately frozen at or below -10 °C or preserved with $HgCl_2$. Mercuric chloride is extremely hazardous and should never be given to volunteers.

Griesbach and Peters (1991) found that filtered samples kept at room temperature could be analyzed for SRP and SP within a week, if stored in the tubes in which they would be eventually analyzed. They did not recommend freezing samples for analysis of the phosphorus fractions. Others report maximum holding times for SRP analysis from less than 24 hours to a week.

Based on the theory discussed above and on the methods, observations, and comments by existing programs, the following recommendations are made.

1. Based on ease of sampling, questions about storage, and utility of the information, total phosphorus should be the primary form of phosphorus measured in volunteer programs.
2. Coordinators should first utilize surface (0.5 m) or integrated epilimnetic samples in their programs, especially if chlorophyll and Secchi depth are to be measured at the same time. A second total phosphorus sample taken in the hypolimnion would add another dimension to the sampling program.
3. The sampling program should have samples taken during spring turnover so that an estimate can be made of the total phosphorus content of the lake. An alternative is to take a detailed vertical profile during summer stratification.
4. Although preservation of total samples is recommended, there are questions about the safety of having the volunteers handle strong acids. Coordinators should consider freezing the samples or having the digestion performed in the sample container itself.
5. Volunteers should be given pre-cleaned, acid-washed sample containers. Manipulation of the sample and the container by the volunteer should be minimized. Phosphorus differences can rapidly set up in a large container as particles settle. The volunteers should be instructed to shake the sample well before pouring. It would be better to pour directly from the water sampling device into the sample container rather than into a second container so that settling in the container and possible contamination is minimized.
6. Soluble reactive phosphorus can also provide valuable information, but requires field filtration through a pre-cleaned membrane or glass fiber filter under clean circumstances. There are also questions of its stability with storage. If done, samples should be rapidly frozen and brought to the laboratory for analysis. Thought should be given to using a syringe filtration system so that the volunteers would not handle the filters.
7. Research should be done as to the importance of the type and porosity of the filter on SRP analysis.

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5.
Temperature and Oxygen

The measurement of temperature and oxygen constitute two of the most basic limnological variables. Each can provide information valuable to the understanding of a lake. Probably both are under utilized in volunteer monitoring programs.

Temperature

Classification

There are a number of basic reasons for measuring temperature in lakes. Temperature is the basis of a thermal classification of a lake. A thermal classification could help the coordinator to separate lakes with similar thermal structures, and, in doing so, perhaps into groups of similar function as well. Thermal structure is a dominant factor affecting many lake processes of interest to limnologists.

A lake can be classified on the degree of thermal structuring during the year. The thermal classification scheme of Hutchinson and Löffler (1956) and Hutchinson (1957) is commonly used. This scheme, summarized in Table 5.1, first divides lakes into those that undergo complete circulation and those that do not.

Holomictic lakes circulate throughout the water column sometime during the calendar year. *Meromictic*

Table 5.1—The thermal classification scheme of Hutchinson and Löffler (1956) and Hutchinson (1957)

Meromictic:	*Lakes that do not undergo complete circulation.*
Holomictic:	*Lakes that undergo complete circulation sometime during the calendar year*
Monomictic:	*Lakes that only circulate once during the year*
Warm monomictic:	*Lakes that circulate only during the winter, having a thermal stratification during the summer*
Cold monomictic:	*Lakes that remain near 4°C throughout the year but circulate only during the summer*
Dimictic:	*Lakes that have a summer stratification and a winter stratification under the ice, circulating only during the spring and fall*
First-class dimictic:	*Lakes that have a bottom temperature near 4°C throughout the year and a strong thermal stratification.*
Second-class dimictic:	*Lakes that are thermally stratified, but the bottom water temperatures rise during summer stratification.*
Third-class dimictic:	*Lakes that are not thermally stratified.*
Polymictic:	*Lakes that stratify irregularly throughout the year*

lakes do not undergo complete circulation: often the lower portion has a chemically induced density difference with the upper waters and is perpetually separated from the overlying water (Hutchinson 1957; Wetzel 1975).

Holomictic lakes are subdivided into *monomictic, dimictic,* and *polymictic* lakes. Monomictic lakes only stratify once during the year. Warm monomictic lakes circulate only during the winter, having a thermal stratification during the summer, while cold monomictic lakes remain near 4°C throughout the year but circulate only during the summer. Dimictic lakes have a summer stratification and a winter stratification under the ice, circulating only during the spring and fall. Polymictic lakes stratify irregularly throughout the year, being either very large lakes in colder climates that have minimal ice cover during the winter (cold polymictic) or lakes in tropical or subtropical regions that stratify irregularly throughout the year (warm polymictic).

Unfortunately, this classification scheme does not adequately address the problem of the temperate shallow lake (Wetzel, 1975), although these lakes are common in North America. They have ice cover during the winter and therefore have a period of thermal stratification. However, during the ice-free season, they may not stratify at all or may have periods of stratification interspersed with periods of free circulation.

Hutchinson (1957) superimposed a second classification on top of the first to account for these unstratified lakes. A *first-class* dimictic lake has a bottom temperature near 4°C and a strong thermal stratification. A *second-class* dimictic lake is thermally stratified, but the bottom water temperatures rise during summer stratification A *third-class* dimictic lake is not thermally stratified. Apparently these unstratified lakes are separated from polymictic lakes in that they do have winter stratification and from monomictic lakes in that they would potentially have two periods of circulation if it weren't for their shallow depths. This designation for the shallow temperate lakes as a third-class dimictic type recognizes their unique thermal properties and the effect of depth.

This classification does more than provide another label for a lake; it also provides insights into how the lake might function. The typical north-temperate dimictic lake (first-class and second-class) has a distinct thermocline during the summer. During that stratified period, oxygen may or may not decline to zero in the hypolimnion. The effects of this anoxia can be profound, depending on the thermal stability of the lake. Stability of a lake is defined as the amount of work needed to mix the entire body of water to a uniform temperature without addition or subtraction of heat (Schmidt 1915, 1928; Hutchinson 1957). This stability can be estimated or indexed by several equations, all of which require detailed thermal information that could be obtained by volunteers. In third-class dimictic lakes, as well as polymictic lakes, the degree of thermal stability is minimal or absent. In this case, the sediments are continually or intermittently exposed to the epilimnetic waters. Oxygen concentrations near the sediments may fluctuate daily, depending on the degree of daily mixing of the epilimnion.

It is also possible that, during the brief periods of thermal stability, that oxygen will disappear near the sediments. During these periods of anoxia, phosphorus may be released from the sediments and build up in the lower strata. When the stratification disappears, this nutrient-laden water will be mixed into the upper water where it may stimulate algal growth. These lakes may be characterized by periodic peaks or pulses in phosphorus and algae throughout the summer ice-free season.

Identification of Seiches

Frequent temperature measurements can also detect the presence of internal seiches in lakes. A steady wind blowing down a lake, a change in air pressure, or a localized storm on a big lake can set the water to rocking with a characteristic period. This rocking of the lake water back and forth is called a *seiche.*

Surface seiches can be detected by changes in water height, but another type of seiche is set up, not at the water surface, but at the thermocline. These internal seiches can rock back and forth for many days. As the water moves back and forth, some of the deeper water that can be rich in nutrients, is mixed into the upper water. Thus the seiche helps fertilize the upper waters and provide the nutrients necessary for algal growth. The effect is accentuated in long, narrow lakes and reservoirs. Either a height recorder or a large number of lake height observations can be used to measure the range and period of a seiche, but frequent temperature measurements at one end of a narrow lake may reveal an internal seiche.

Quantification of Thermocline

Probably most volunteer programs simply plot temperature over time or depth without considering that there is a wealth of other valuable information available that is being ignored. Once the data is entered into a spreadsheet, it is possible to quantify the depth of the thermocline and even the depth of the top and bottom of the metalimnion. This information can be used to alter sampling depths if sampling depends on thermocline depth or depth of the epilimnion. The information also makes an easily interpretable graph of how and when the thermocline sets up and declines.

If you calculate the rate of change in temperature,

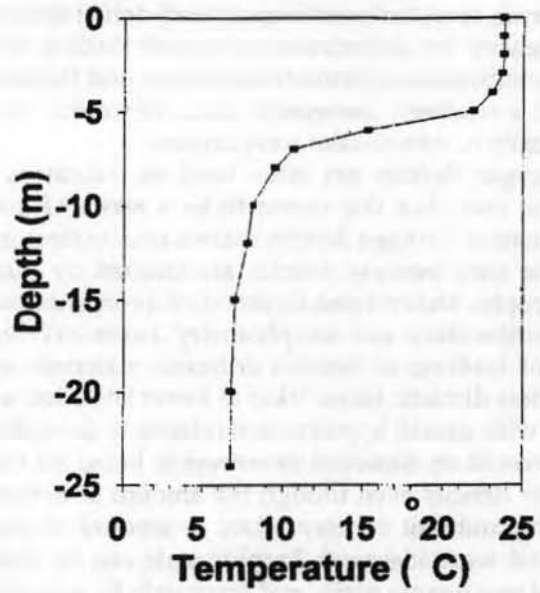

Figure 5.1: A temperature profile

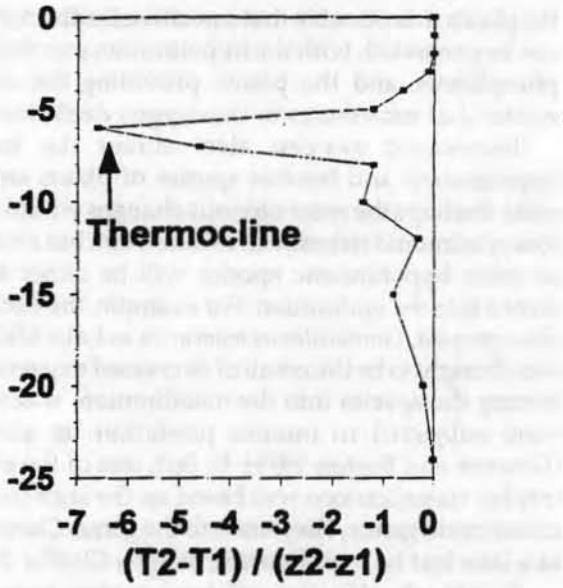

Figure 5.2: Location of a thermocline

the thermocline is defined as the point of maximum rate of change (Figs. 5.1 and 5.2). This is easily calculated on a spreadsheet by taking the difference in temperature at two consecutive depths. This difference is divided by the difference in depth. The resulting value is the rate of change in temperature.

The top and bottom of the metalimnion are defined as the minimum and maximum of the second derivative of the change in temperature (Fig. 5.3). These values are easily calculated as the rate of change of the rate of temperature change calculated above; subtract the rate of change in temperature at two consecutive depths and divide this difference by the difference in depth. With these values, you can easily plot the seasonal change in the depth of the thermocline and the width of the metalimnion.

Oxygen

Hypolimnetic oxygen concentration has long been considered to be an important indicator of eutrophication. With increased nutrient concentrations in the epilimnion and the subsequent increase in plant biomass, the amount of organic material injected into the hypolimnion increases as well. In a stratified lake, these increased organic loads increase the decomposition rates and, subsequently, the rate of oxygen depletion. The depletion of oxygen from the hypolimnion can cause a number of significant changes in the chemistry and biology of a lake. The loss of oxygen will be accompanied by lower oxidation-reduction potentials in the bottom waters, and the appearance of a number of soluble reduced compounds, including iron and manganese.

If phosphorus, previously bound to iron hydroxy complexes, is released, it may find its way through the thermocline, providing a potentially significant internal source of phosphorus to the epilimnetic plants. With an internal source of phosphorus provided for

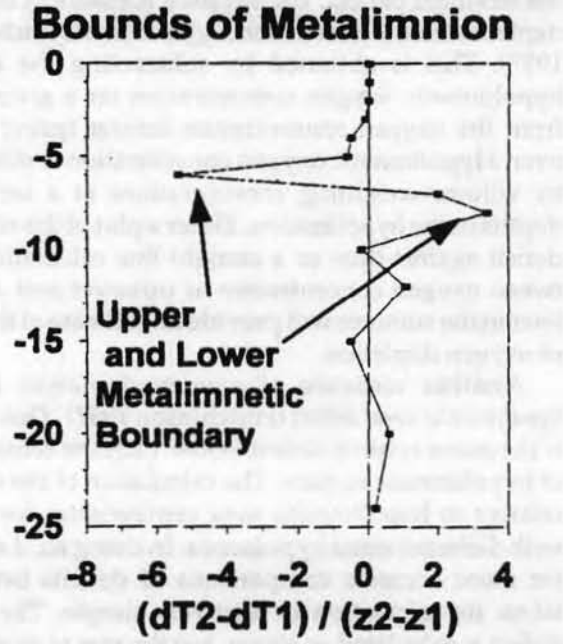

Figure 5.3: Bounds of the metalimnion

the plants, it is possible that a positive feedback system can be generated, with the hypolimnion providing the phosphorus, and the plants providing the organic matter that contributes to the oxygen depletion.

Decreased oxygen also causes the loss of hypolimnetic and benthic species of plants and animals. Perhaps the most obvious changes would be the loss of salmonid fishes such as lake trout, but a number of other hypolimnetic species will be either lost or forced into the epilimnion. For example, the decline of the copepod, *Limnocalanus macrurus*, in Lake Michigan was thought to be the result of decreased oxygen levels forcing the species into the metalimnion, where they were subjected to intense predation by alewives (Gannon and Beeton 1971). In fact, one of the earliest trophic classifications was based on the shift from the chironomid genus, *Tanytarsus* to the genus *Chironomus* as a lake lost its hypolimnetic oxygen (Rodhe 1975).

Trophic classification of lakes has often been made solely on the presence or absence of oxygen in the hypolimnion, but this method is subject to error because the oxygen does not deplete immediately upon thermal stratification; the depletion rates depend, not only on the organic load, but the oxygen concentration during turnover, the temperature of the hypolimnion, and the morphometry and size of the hypolimnion relative to the size of the epilimnion (Hutchinson 1957). The presence or absence of oxygen will also depend on when the hypolimnion is sampled relative to the time of stratification.

The rate of oxygen depletion is a more useful measure than presence/absence information, but does require samples to be taken periodically throughout the stratified period. The simplest measure of oxygen depletion rate is the *relative oxygen deficit* (Hutchinson 1957). This is obtained by subtracting the actual hypolimnetic oxygen concentration on a given day from the oxygen concentration during spring turnover. Hypolimnetic oxygen concentration is obtained by volume-weighting concentrations at a series of depths in the hypolimnion. Either a plot of the relative deficit against time or a straight-line calculation between oxygen concentration at turnover and a date later in the summer will provide an estimate of the rate of oxygen depletion.

Another measure of oxygen depletion is the *hypolimnetic areal deficit* (Hutchinson 1957). This index is the mean relative deficit below 1 square centimeter of hypolimnetic surface. The calculation of the deficit relative to hypolimnetic area compensates for lakes with different sized hypolimnia. In doing so, it allows for more accurate comparisons of deficits between lakes. Its calculation is relatively simple. The areal deficit is calculated as above, but the rate of depletion is then divided by the hypolimnetic area (cm^2). An example of its calculation is given in Wetzel (1975). However, even the hypolimnetic areal deficit does not compensate for differences in organic loading from humic substances or from temperature, and therefore is still a relatively inaccurate index of trophic state, especially in lake-to-lake comparisons.

Oxygen deficits are often used as indicators of trophic state, but this seems to be a waste of good information. Oxygen deficits make a poor indicator of trophic state because deficits are affected by many non-trophic state related factors such as organic loading, temperature, and morphometry. Lakes with high organic loadings of detritus or humic materials, second class dimictic lakes, lakes at lower latitudes, and those with a small hypolimnion relative to the epilimnion would be classified as eutrophic based on their oxygen deficits even though the amount of nutrient loading, nutrient concentration, or amount of plant material were identical. Trophic state can be determined much more easily and accurately by a number of other variables.

However, the rate at which oxygen disappears from the hypolimnion is an important piece of information both to understand the dynamics of a lake and for its management, especially if that rate is quantified and tracked from year to year. Oxygen deficits are most often used to compare different lakes, but differences in morphometry, geographic location, and trophic state can make the comparisons less useful. However, if yearly measurements are made on the same lake, then morphometry and location become constants in the relationship.

One would expect that, as a lake eutrophies, the rate of oxygen consumption in the hypolimnion would increase. This would happen for two reasons. First, because more algae and macrophytes would be produced in the epilimnion, it would be expected that more organic material would settle into the hypolimnion and decompose there. Second, some of the material would not be completely decomposed and would settle onto the sediments. In the following years, the decomposition of the organic sediments would also place a demand on the oxygen supply of the hypolimnion. The initial disappearance of oxygen in the hypolimnion can occur prior to any noticeable change in the productivity of algae in the epilimnion because of the amplification of organic epilimnetic inputs by the sediments (Gliwicz and Kowalczewski 1981). This makes the oxygen content of the hypolimnion and the rate of disappearance to be a potential "early warning system" of changes in trophic state.

Temperature Measurement

A number of methods exist for measuring temperature, with costs ranging from a few dollars to several hundreds of dollars. The simplest method requires

only an accurate thermometer, preferably one with a metal or plastic shield that minimizes the possibility of breakage. When a thermometer is used, water has to be brought up from the desired depths, the thermometer inserted, and, after a period of equilibration, the temperature read. Care must be taken so that the temperature does not change during the time from sampling to the time the temperature is read. This might be accomplished by rapidly bringing the water up from the depth, using an insulated or thick plastic sampling container, and minimizing the time of exposure of the enclosed sampler to the ambient air temperature.

Another method for taking this reading is to have the thermometer permanently attached in the sampler, either at the top or fixed to the inside of a clear sampler. In this case, all the volunteer has to do is take the sample, bring it to the surface, and read the thermometer. Again, the coordinator must be certain that the apparatus is constructed in such a manner that the temperature of the sample does not change significantly during its assent.

A second method to obtain temperature information is to drop a weighted maximum-minimum thermometer to the desired depth (Lind 1985). The maxmin thermometer is constructed in such a way that the maximum temperature (air or surface water) and the minimum temperature (the temperature at depth) will be recorded. If the lake is "normal," and temperatures decrease continuously with depth, a series of measurements of the minimum temperatures should accurately reflect the temperature profile of the lake.

The most convenient method for measuring temperature is using an electric thermistor thermometer. This device works on the principle that temperature changes the resistance of a wire to an electric current. In these instruments, the only sensitive portion of the wire is the tip, or probe. The wire is simply lowered to the desired depth, the probe allowed to equilibrate, and the temperature measurement taken. Although the instrument is convenient, especially when coupled with an oxygen probe (see next section), it is important to calibrate the instrument against an accurate laboratory thermometer over the entire temperate range encountered in the lake.

It is also important that batteries of the thermistor not be allowed to decline in output. If the instruments are calibrated and fresh batteries installed in the spring, it may be that their accuracy will remain high throughout the summer season, but the coordinator should be sure that this is the case. Thermistor instruments range in costs from $40-50 for fishermen's models up to several hundred dollars for scientific instruments. It might be expected that accuracy varies proportional to cost, but this needs to be examined.

Oxygen Measurement

As with temperature, several methods exist for measuring oxygen, with wide-ranging costs. Considerations include convenience, time, accuracy, and safety. The least expensive method involves the chemical determination of oxygen in a water sample. Water has to be brought up from the desired depth, a known volume poured into a container, reagents added, and, finally, the sample titrated. Although limnologists usually do this procedure in the laboratory, several companies make relatively simple field test kits that would allow a volunteer to perform the test. What is sacrificed with these test kits may be accuracy, because, while the test is similar to that used in most limnological laboratories, the titration burette used in the laboratory may be replaced by an eye dropper or a syringe. While accuracy may be sacrificed, the loss may not be that important to a volunteer program, where the presence or absence of oxygen, not its concentration, accurate to the first decimal place, may provide adequate information.

A more important consideration with chemical tests is that of safety. These chemical techniques involve the use of strong acids and bases. The coordinator must decide whether there is risk in allowing volunteers to use potentially deadly chemicals without supervision, especially if children may be in the volunteer household. Certainly adequate precautions should be taken to educate the volunteers on the potential dangers of the chemicals and to provide a child-proof box in which to store the chemicals.

The alternative to chemical analysis is convenient but expensive. This technique involves using an oxygen probe that is lowered to the desired depth and the oxygen concentration directly read off a meter. An oxygen probe generally uses a reducing electrode covered with an oxygen-permeable membrane. Oxygen passing through the membrane is reduced at the electrode, and the resulting current is measured (Golterman and Clymo 1971).

The dissolved oxygen probe is relatively accurate at high and medium oxygen concentrations, but takes increasingly longer times to equilibrate at very low oxygen concentrations, and may indicate slight amounts of oxygen present when there is actually no oxygen. The membrane on the probe needs care and must be replaced periodically; it must also be calibrated against an atmospheric standard each time it is used. Periodic calibrations against oxygen concentrations determined chemically should also be done to check for accuracy and linearity.

Another method of indirectly determining oxygen depletion would be to suspend a copper chain into the

water. As the oxygen is depleted and a reducing environment is produced, reduced sulfur will be found in the water. This sulfur will combine with the copper to produce copper sulfide, a black precipitate on the chain. In Carlson's experience it takes several hours for a noticeable color change to take place. Volunteers could permanently suspend a chain in the water and then periodically check the extent of a blackened color on the chain. This technique should be considered as a possible surrogate for more expensive and hazardous methods for estimating the rate of oxygen depletion.

Methods Used by Programs in the Upper Midwest

The Ohio Citizen Lake Improvement Program has volunteers take a surface water temperature. The Wisconsin Self-Help Lake Monitoring Program trains their volunteers to take temperature and oxygen profiles.

Ohio Citizen Lake Improvement Program

Temperature is measured with a small thermometer encased in a metal protective shell. A polyethylene cubitainer is lowered one foot below the surface and a sample of water taken. The thermometer is inserted through a slit in the cubitainer, left for two minutes, and then the temperature read while the probe remains in the sample. Only surface water temperatures are measured in this program. Oxygen is not measured in this program.

Wisconsin Self-Help Lake Monitoring Program

A temperature profile is used in Wisconsin to locate the thermocline. In 1990, a Taylor Instruments mercury/glass pocket thermometer was used, but there was a great deal of breakage. These were replaced with stainless steel, dial-face thermometers made by VWR. Both types of thermometers were calibrated in the laboratory against a liquid-in-glass thermometer certified by the National Bureau of Standards (Betz et al. 1990). Currently volunteers use a non-mercury liquid thermometer attached inside the sampling bottle.

Volunteers measure oxygen using a chemical analysis procedure (LaMotte Chemical). According to Betz, et al. (1990), the kit contains sulfuric acid to preserve the sample, modified Winkler chemicals (manganous sulfate, alkali-iodide-azide) to convert the oxygen to iodine, and finally a direct reading titrator for the titration of the solution against a sodium thiosulfate solution. (Currently sulfuric acid liquid is used in place of the sulfuric acid powder.)

Starch is used as the indicator. Betz (1992) reports that the kits were modified to produce more accuracy and sensitivity. A small graduated cylinder and a eyedropper were added to more accurately measure sample volume. A second change was the addition of a 20 gauge hypodermic needle (with the tip filed down) to the glass syringe used for titration. The original syringe gave oxygen concentrations with an accuracy of ± 0.6 mg/L; the additional needle increased the accuracy to 0.1 mg/L .

Program officials work with the volunteer to establish appropriate interval distances for the profile. In many lakes, the interval distance is three feet. The volunteer uses the horizontal point sampler to collect the water sample at each successive interval. The sample is brought to the surface and the temperature is read from the thermometer mounted in the sampler. Then a portion of the sample is collected in a special bottle used in the measurement of dissolved oxygen (D.O.) concentration.

Listed below are the instructions for measuring the temperature and dissolved oxygen profile. All the equipment and supplies mentioned have been provided to the volunteer. The instructions are generalized from the training manual given to Wisconsin volunteers.

Instructions for On-The-Water Activities

- Prepare the water sampler and collect a water sample from the near surface depth.
- Raise the sampler into the boat and record the water temperature on your data sheet at the appropriate depth. (Give the thermometer a few minutes to register)
- Remove the cap of the appropriate D.O. bottle.
- Insert the rubber hose of the sample to the bottom of the D.O. bottle. Open the hose clamp and allow water to flow into the D.O. bottle, overfilling the bottle at least three times.
- Slowly remove the tube allowing the D.O. bottle to overfill.
- Cap the D.O. bottle
- Put on your plastic gloves and safety goggles.
- Remove the D.O. cap again and add eight drops of the Manganous Sulfate Solution from the squeeze bottle.
- Add eight drops of the Alkaline Potassium Iodide Azide Solution
- Cap the D.O. bottle and mix by inverting the bottle ten to twenty times. Put the bottle in the small tray and allow the precipitate (the solid substance that is now forming in the bottle) to settle.
- Once the precipitate has settled half way down the bottle, invert the bottle another ten to twenty times to remix the precipitate. Let the precipitate settle again.

[The next step may be done on the shore, if desired]

- Once the precipitate has settled for a second time, add eight drops of Sulfuric Acid from the squeeze bottle. Cap the D.O. bottle and invert it ten to twenty times to mix.
- Repeat the above steps for each successive depth you are sampling.

Instructions for On-Shore Activities

- Set up your dissolved oxygen kit in a place that is convenient to work.
- Take out the 25 ml graduated cylinder and rinse it with distilled water. Also rinse the small glass vial with the plastic lid and center hole.
- Take out the first "fixed" dissolved oxygen sample from the 1-foot depth. Uncap the D.O. bottle and fill the graduated cylinder to the 20 ml line with the "fixed" water sample from bottle no. 1. When you look at the water from the side, you will see the water surface sag downward. Keep pouring until the bottom of this sag lines up with the 20 ml line. The graduated cylinder will give a far more accurate measure of volume than pouring directly into the small glass vial.
- Transfer the 20 mls of fixed D.O. solution from the graduated cylinder to the small glass vial.
- Insert the syringe into the Sodium Thiosulfate Solution. Turn the bottle and syringe upside down and very slowly draw up the solution past the line marked "0". Remove any air trapped in the syringe by pushing the liquid back into the bottle several times until the bubbles are expelled.
- Attach the hypodermic needle to the syringe and remove the needle cap. Expel extra Sodium Thiosulfate to the line marked "O" by pushing on the plunger of the syringe.
- Insert the syringe into the hole of the vial cap. Add the Sodium Thiosulfate Solution very slowly by pushing on the plunger of the syringe. Gently swirl the glass vial after each addition. It is possible to add as little as 0.1 unit (half the distance between the lines on the syringe) with each addition.
- Continue adding the Sodium Thiosulfate Solution until the color of the water sample has changed to a very faint (straw) yellow. The amount of the Sodium Thiosulfate that you add will vary between samples depending on the amount of dissolved oxygen in the sample. (Note: The exact color is not that important. The object is to add drops to lighten the color, but to stop before the solution becomes clear.)
- When you have achieved the straw yellow color, carefully remove the syringe from the vial and set the syringe aside. DO NOT EMPTY THE CONTENTS OF THE SYRINGE! (If the dissolved oxygen content of the water is great, the water may not have become a faint yellow color even after you have added the entire contents of the syringe. In this case, you will need to refill the syringe with the Sodium Thiosulfate solution by repeating steps 3 & 4. Make sure you note this on your data sheet.)
- Remove the plastic cap with hole from the glass vial.
- Fill the medicine dropper with the Starch Indicator Solution. Add 8 drops of this solution to the 20 ml glass vial. Mix gently by swirling the vial. The sample will turn blue.
- Replace the plastic cap with hole onto the 20 ml glass vial. Reinsert the glass syringe, (which should still have Sodium Thiosulfate in it from step 5), into the glass vial.
- Continue, as you did before, to add the Sodium Thiosulfate in very small increments until the blue color in the vial just disappears (solution will turn clear). Don't go too fast! Take your time to swirl the contents of the glass vial as the color disappearance may take a few seconds. Disregard any blue color that reappears after the solution clears.
- When the solution has turned clear, remove the glass syringe. Before expelling the remainder of Sodium Thiosulfate solution in the syringe, read and record the volume of Sodium Thiosulfate that was used. This step is very important as this is the "answer" to the dissolved oxygen content of that water sample! Once you have recorded the amount used, you can discard the extra solution by flushing it down the drain with lots of water.
- Rinse the needle with distilled water and wipe it off before moving onto the next sample.

If the volunteer makes a mistake, there is enough sample water to do the on-shore procedure twice. The equipment is cleaned and rinsed with distilled water. The original data sheets are then forwarded to program officials.

Quality assurance in the oxygen determinations is obtained in two ways in the Wisconsin program. Volunteers air saturate a pint of distilled water and titrate three aliquots of the sample. Knowing the temperature and altitude, the program coordinators compare the volunteer's results with the expected oxygen concentration at saturation. Second, the volunteer oxygen profiles are compared to those done by the agency using either standard Winkler titrations or electronic meters (Betz 1992).

Other Programs

New York

In the New York program, temperature is measured using a combined temperature-oxygen meter (Nester field D.O. meter). According to Kishbaugh and Saltman (1992) this meter is less sensitive to "unfriendly field and operator conditions" and minimizes probe and membrane maintenance.

One meter is shared by a group of neighboring lake associations (4-6 lakes per meter). Temperature and oxygen are measured at 1 meter intervals to approximately 1 meter above the bottom (as determined using the Secchi disk). The lake associations are responsible for the daily maintenance of the meter, including air calibrations and probe care. The lake associations transport the meter to the next group who will be using it (Kishbaugh and Saltman 1992).

Recommendations

1. If economically possible, temperature and oxygen measurements should be made a part of the monitoring program.
2. Temperature equipment is not necessarily expensive, and can be economically incorporated in programs.
3. Oxygen measurement is more difficult, potentially dangerous, and expensive. If chemical techniques are to be used, considerable attention should be made to the training of the volunteers, warning them of the potential hazards of the chemicals being used. The sharing of a single instrument among several groups, as is done in the New York program may be an economical solution.
4. Effort should be made to extend the use of the information gathered beyond simple time-plot graphs. Temperature and oxygen profiles are integral to the understanding of a lake's dynamics, and the data should be used to a maximum extent in gaining that understanding.

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6.
Sampling Procedures

When limnologists think about sampling design and sampling statistics, it sometimes seems that these thoughts are perfunctory. Statistics seem to become a concern only after the project is completed and the researcher tries to make some sense of the collected data. Despite numerous warnings from statisticians and limnologists with statistical training, statistical considerations seem to be the last concern of projects rather than the first.

In volunteer monitoring projects, statistics may receive even less consideration because the control of lake, site, date, or even technique is removed from the coordinator's control. The coordinator is faced with the dilemma of obtaining meaningful data despite the variability inherent in volunteer monitoring. The bottom line is that every reasonable effort in care, design, transport, handling, and sampling should be taken. High quality data will greatly facilitate all subsequent predictive and diagnostic efforts.

This chapter cannot be a complete review of sampling statistics, nor is another such review necessary. A number of manuals as well as statistics books exist that can give the coordinator information on sampling design. Manuals such as Gaugush (1986, 1987), Reckhow (1979), Reckhow and Chapra (1983) specifically deal with the sampling of lakes and reservoirs.

Bias: What Will Compromise Data

Statistics books put a great deal of emphasis on the concepts of accuracy, precision, and bias. Accuracy is the closeness of a measured or computed value to its true value, while precision is the closeness of repeated measurements of the same quantity (Sokol and Rolf 1981). Bias, as described by Standard Methods (APHA 1989), is a systematic error introduced into the estimate by either the method (site selection, sampling, storage, or analysis) or the use of the method (how the volunteer or the laboratory interprets the method). The goal of any sampling program should be to have low bias, high precision, and high accuracy.

Precision is maximized by careful instructions to the volunteers, a standardized sample collection and storage procedure, minimization of the need for the volunteer to handle samples, and, of course, careful analytical laboratory procedures. Quality assurance (QA) is the set of operating principles erected at the beginning of the program to guide both the volunteer and the laboratory in this pursuit of precision. Once a quality assurance plan is formulated, then quality control (QC) procedures can be developed and used to ensure that the volunteer and the laboratory are maintaining the necessary degree of precision.

After the coordinator has done all that is possible to train the volunteers in sample collection, handling, and storage and is assured that the laboratory can do the analyses carefully, then the problem will lie with the reduction of bias. Bias is perhaps the most insidious of the possible errors in a sampling/analysis program because it is the unseen, the unplanned, the unintentional error that sneaks into the program when everything seems to be so well-planned

Bias is that systematic error caused by a laboratory carefully, precisely, and with appropriate quality control procedures, using the inappropriate analytical procedure for total phosphorus or chlorophyll. Bias is

the volunteer carefully, precisely, and with several replications, measuring Secchi depth in a bed of weeds. Bias is having a colorblind volunteer estimating the water color. Bias is depending on the data from volunteer-chosen lakes, rather than randomly chosen lakes to be used as an indicator of a state's lake water quality. Bias is the error that can invalidate the data of the most well-planned monitoring program.

Bias can be dealt with in two very different ways. The first technique attempts to eliminate the bias. The second, acknowledges that bias exists and shapes the expectation and interpretation of the data accordingly. Both techniques can be employed in volunteer monitoring programs. The best, and most difficult, method to deal with bias is to try to eliminate it. A great deal is known about sampling and analytical bias, and an informed coordinator can minimize the bias. Much of the discussion below deals with identifying forms of bias describing methods that can eliminate it.

However, sometimes bias cannot be easily removed. In some instances, the roadblock to the elimination of bias is the cost of doing so. Characterization of a lake's water quality may require samples taken at numerous locations in the lake, but the cost of doing so would be too large. In this case, the coordinator must deal with the problem of too few samples at too few locations.

In other instances, it is known that laboratory procedures produce data that is highly dependent on the method used. Accurate data may cost too much or may be impossible to attain under any circumstance. How does the coordinator deal with analytical bias?

One of the most helpful techniques for maximizing the collection of accurate and useful data is to begin with a careful and thoughtful consideration of the goals of the project. Goals certainly help to define the expectations of the project, but, more importantly, also guide the coordinator in choosing appropriate sampling strategies. In some cases, the goals might suggest that a volunteer program is not appropriate. Once goals are formulated, then the methods to best realize these goals can be devised. The possible biases accompanying each method can be listed and either eliminated, minimized, or managed.

Sampling Procedures

There are three commonly used sampling strategies: random, stratified random, and sequential. A random sampling procedure requires that the investigator generate a sampling scheme using a random selection of the sample. The details of these techniques are described in many statistics books, and will not be redescribed here.

In limnology, a truly random sample would require a random selection of site, time, date, and/or depth (Table 6.1). A random sampling procedure is the simplest to analyze, but it does not account for known gradients within lakes, depths, or geographical regions, which may have differing mean values and variances.

If gradients in site, time, date, depth, or region do occur, it might be best to examine parts of the gradient separately. To do this it is useful to use a stratified random sampling procedure. In this procedure, the

Table 6.1.—Possible sampling strategies for lakes and reservoirs.

	Random	Stratified	Sequential	Typical Limnological Sampling Strategy*
Lake	Randomly chosen from geographic area	Randomly chosen from within a geographic region (state, county, ecoregion) or some other classification (recreational, water supply)	Sample every lake along a transect, using a randomly chosen transect starting point	Choose lake based on convenience, access, proximity, or interest
Site	Randomly chosen from lake grid	Randomly selected from within regions of lake	Sample at pre-chosen, equidistant sites along transect of lake, starting with a randomly chosen point	Sample at the dam or over the deepest part of the lake
Depth	Randomly chosen	Randomly chosen within depth regions (epilimnion, hypolimnion, photic, etc.)	Sample at preset interval starting with a randomly chosen depth within the first interval	Sample at the surface or at preset intervals, surface to bottom
Date	Randomly chosen	Randomly chosen within season, month, or limnological period	Sample every two weeks, starting with a randomly chosen date	Sample the same day every week
Time	Randomly chosen	Randomly chosen within period such as daylight or some other division of day	Sample every two hours starting with a randomly chosen time	Sample when you get there

*The "Typical Limnological Sampling Strategy" is not necessarily recommended by the authors

pool of all possible samples (lakes, locations, or dates) is split into smaller, more homogeneous entities. A state may be divided into ecoregions, a lake divided according to areas such as main lake or embayment, depth into epilimnion and hypolimnion, and the year into seasons. Within each of these divisions, samples are then taken randomly.

A third sampling procedure is sequential sampling. In this case a starting point is chosen randomly, and samples are then taken at set intervals (distance, depth, or time) from that point (Cochran 1977). Sequential sampling may or may not produce more accurate estimates than do random sampling, but in limnological sampling it is often the easiest. However, it does have several drawbacks.

First, there is no simple estimate of standard error (Cochran 1977). Second, samples drawn in sequence may be autocorrelated; the next sample in the series might be predicted to some extent by the preceding sample. Autocorrelation poses a problem because samples are not drawn independently and randomly from a pool of possible variable, and therefore, the statistical number of samples being taken is less than the actual number of samples (Cochran 1977; Reckhow and Chapra 1983). Autocorrelation may affect random and stratified sampling procedures as well.

A third drawback of sequential sampling is that, as illustrated if Fig. 6.1(A) if there are periodic fluctuations in the variable of interest, it is possible with sequential sampling to always sample the same part of the curve (Cochran 1977). For example, chlorophyll may rise and fall regularly during a 24 hour period because the algae migrate towards the surface at night and descend during the day. If surface samples were taken every day at noon, the chlorophyll concentrations in the lake would be underestimated. If they were taken at midnight, the estimates would be exaggerated. If a single yearly sample was taken in December or June or August, different estimates of annual chlorophyll might be found. As illustrated in Fig. 6.1(B), samples should be taken at a frequency different from the suspected repetition frequency, so that over time, they sample all portions of the periodic function. For example, if weekend boat traffic affects transparency, select a sampling frequency of six or eight days instead of seven-day intervals.

In Fig. 6.1 (C), a random sampling design is also illustrated. In this case, an excellent estimate of the mean might be obtained over the course of several periods, but certain portions of the period may be missed. If this curve represented an annual chlorophyll curve, in some years there would be no samples taken at the highest months, thus under-representing chlorophyll in those years. An alternative to either the sequential or completely random design would be to use a stratified design. This design would allow for each period to be partitioned into relevant smaller periods, yet still provide the statistical rigor required.

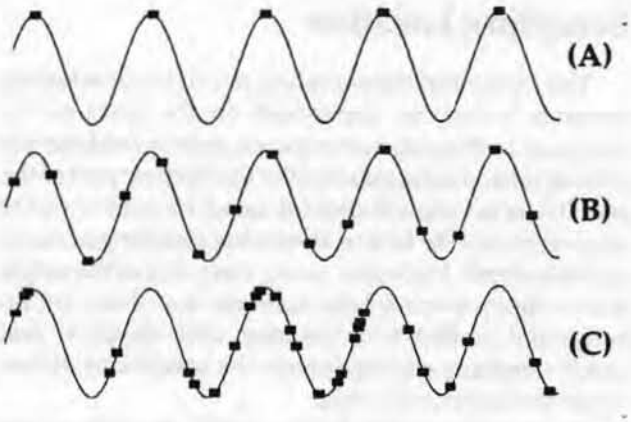

Figure 6.1—Graphic representations of sequential (A) and (B), and random (C) sampling designs.

What Lakes to Sample

Bias can creep into a monitoring program when lakes are chosen for the program. If the program purpose is to provide information on, for example, the trophic state of the state's lakes, then the lakes of the state must be sampled without bias; every lake must have the equal potential to be included in the program. The coordinator might wish to stratify the sampling within geographic, political, or ecological regions within the state, but, within each division, the lakes still should be sampled randomly.

Rarely do volunteer monitoring programs have the luxury of choosing volunteers on randomly selected lakes. More often, the lakes are selected based on the availability of volunteers. It is probable that the lakes will not be chosen randomly and, therefore, may produce data that will not be unbiased estimates of the state's lakes. For example, volunteers may be more available for recreational lakes, for lakes where they have cabins, or for lakes that are easily accessible. Private lakes, lakes in state parks, small, shallow, or weedy lakes may be under-represented.

A solution to this dilemma is to reexamine the goals in light of the realities of the volunteers and their lakes. The coordinator could either randomly match chosen lakes with volunteers and fill in the non-volunteer lakes with agency staff or change the goals of the program. Perhaps expectations could be modified so that the recreational lakes of the state are adequately sampled. As a last resort, a coordinator can just accept the fact that the program estimates the trophic state of a potentially biased set of volunteer-sampled lakes. The important consideration may not be that the data are biased, but how the data are interpreted and used once they are gathered.

Sampling Location

The location of the sampling point in a monitoring program is heavily dependent on the goals of the program. In the past, many limnologists would sample natural lakes at a single site over the deepest part of the lake. There is a good reason for using the point over the deepest part of the lake; it allows the sampling of every possible depth. However, using this point as the single site to characterize a lake assumes that there are no horizontal gradients in the lake; each depth at that single sampling site represents the conditions at that depth throughout the lake.

As emphasized by Peters (1990), the use of the single sampling site also ignores the characterization and influence of the littoral zone, which can constitute a significant percentage of the total lake area and volume. Peters also suggests that marked horizontal differences should occur over horizontal distances of only 1500 meters (0.9 miles) in a 15 meter deep lake. Imagine the immensity of the influence of this single point, deep water sampling procedure has had on our perspective of lakes. This procedure not only rejects the possibility of spatial gradients in the lake, but ignores the littoral zone entirely, both as a component and its functional role in the lake system.

The characterization of reservoirs adds an entirely new dimension to the problem of selecting a sampling site. Many reservoirs are long and narrow, with a majority of the water flowing into the reservoir at a single point. Others are highly dendritic, with many bays and inlets, often with their own contributing stream. Spatial differences in water quality in reservoirs are the rule, not the exception, and assumptions about homogeneity at a given depth simply cannot be made. To attempt the characterization of the entire reservoir by a single sample would be almost meaningless.

Two different approaches have been suggested to characterize heterogeneous bodies of water. The first, recommended by Thornton, et al. (1982) and Gaugush (1987), suggests that sampling stations should be more numerous where concentration gradients are the highest, such as near stream and river inflows. Before initiation of a sampling program, the lake or reservoir would be intensively sampled. Replicate surface samples are taken at each site, and a statistical multiple range test used to identify significant differences between station means. If the means between two different stations are not significant, then only a single sampling station would probably be sufficient. Where station differences are significant, separate stations would be established.

In contrast, Walker (1987) suggests that sometimes these areas of greatest change, such as near inputs and in narrow embayments, do not constitute a large proportion of the reservoir's volume. More emphasis should be placed in sampling the areas of a reservoir where most of the volume can be found. Walker's approach would concentrate on sampling the bulk of the reservoir's volume. These two different methods of sampling reflect the need to characterize the entire surface area of the reservoir (or lake) versus the need to represent the condition of the bulk of the water body. The first approach might be better for characterization, while the second for producing an accurate estimate of the content of a material, such as phosphorus.

A third approach does not require such extensive sampling effort. If the goal of the program is to monitor changes in water quality over seasons and years, then complete characterization of the lake or reservoir may not be necessary. In this case, sampling at a single site might be appropriate. The important factor is that the site be clearly marked, so that anyone, not only the initial volunteer, can find the site again. The sites could be documented, for example, using triangulation with permanent shoreline markers, such as houses, water towers, etc. or a satellite-based locating device to establish the exact latitude and longitude. Several sites should be monitored on large or dendritic lakes in order to keep track of local changes.

Depth

Limnologists use one of several methods when sampling with depth. They may sample only at the surface or they may take a series of samples at pre-set intervals from the surface to the bottom of the lake (0 m, 1 m, 2 m, etc.). Usually a "surface" sample is taken just below the surface (0.5 meters or 1 foot) to avoid surface scum. A third approach is to take a single integrated sample with a tube or hose sampler from the surface to some predetermined depth (euphotic depth or thermocline). None of these techniques is entirely satisfactory, either because of limnological, logistical, or statistical considerations.

The advantage of a single sample taken at the surface is that it requires only one analysis, an important consideration to a program on a budget. It also does not require any special sampling apparatus; the volunteer can just hold a sampling container under the surface of the water. Because it is simple and inexpensive, it is an attractive sampling technique for volunteer programs.

Taking a single sample at the surface assumes that the lake, or at least the epilimnion, is completely mixed, and the surface sample is an adequate estimator of the lake or epilimnetic concentration. In many cases, the assumption of a completely mixed body of water is incorrect. Many lakes have vertical gradients of temperature, oxygen, nutrients, and algae that will be missed by the surface sample. Most certainly, deeper

lakes establish a thermal gradient in the summer and the lake will have at least two thermal regions, and, possibly, at least two chemical regions as well. Even in shallow, usually well-mixed lakes, chemical gradients will develop during calm periods. Gradients can even occur in the upper waters of lakes.

Metalimnetic populations of algae will develop in many stratified lakes (Hanna and Peters 1991; Knowlton and Jones 1989). Blue-green algae also migrate deeper into the epilimnion during the day, and a surface sample would miss them. On the other hand, on calm days, blue-green algae may float to the surface, forming surface scums. If sampled on these days, the amount of phosphorus and chlorophyll would be highly exaggerated.

Sampling at intervals through the entire water column is the standard alternative to the single surface sample. If sampled at sufficiently close intervals, the technique detects gradients within the water column. The technique is also used when constructing a nutrient budget to estimate the total content of a nutrient, such as phosphorus, in the water column.

As it is commonly done, samples are taken at fixed intervals, often beginning at the surface and proceeding at fixed depths to the bottom. However, this type of interval sampling violates one of the requirements of sequential sampling—it is usually started at the same depth each time. Sequential sampling requires that the starting depth be randomly selected. For example, sampling might be chosen randomly to begin at 0 meters on one day and 0.4 meters on the next visit.

If a randomly chosen starting point is not selected each time, there is the possible introduction of bias into the estimate. For example, the predetermined sampling points might continue to miss a thin, metalimnetic algal population or a near-sediment phosphorus or oxygen gradient. Interval sampling also requires a larger number of analyses than the single sample. The coordinator should consider whether the information gained, or the error minimized, by interval sampling can be justified by the added expense. If the values are to be used in an estimate of the total concentration in the lake, then the random starting point may be necessary. If the purpose of the sampling is to document changes in concentration at fixed depth, than a fixed starting point would be justified.

The hose or tube sampler collects and integrates a column of water into a single sample. The volunteer lowers the weighted end of a plastic hose (even garden hose has been used) to a given depth. The other end of the hose, which is in the boat, is then corked. The weighted end is pulled to the surface by an attached line. If the top end of the hose is well sealed, the water will remain in the hose as it is raised. Once the weighted end is in the boat, it is placed inside a sample container, the other end uncorked, and the entire volume of the hose emptied into the container. A rigid tube can be used instead of a hose, but a longer tube becomes cumbersome to lift into the boat, and it is difficult, especially for a single person, to pour the sample into a bottle.

Like the interval sampling technique, a greater proportion of the water column is included in the hose sample than is obtained with the surface sample, but unlike interval sampling, the water is mixed together in the hose or sampling container, producing a single sample. A single sample is less costly, but the information about possible gradients is lost as well. The supposed advantage of the hose sample is the possibility that it better represents the actual concentration of the target variable within the water column than does the surface sample. It is also less expensive, both in terms of expense of equipment and of number of analyses, than interval sampling.

The hose sample does suffer from several disadvantages. Someone, probably the volunteer, is going to have to decide the maximum depth to which the sampler will be lowered. The hose could be lowered to the bottom of the lake, to the bottom of the epilimnion, or to the bottom of the euphotic zone (1% light). None of these depths is fixed from lake to lake nor necessarily constant from visit to visit. The volunteer would have to make a decision about the depth prior to lowering the sampler.

If the hose is to be lowered to the bottom of the lake, the volunteer would have to estimate the depth of the lake without disturbing the sediments, perhaps using the Secchi disk as a depth indicator. Any sediment disturbed by the disk or the sampler itself would invalidate the sample. If the hose is to be lowered to the thermocline or bottom of the epilimnion, the volunteer would have to estimate either the depth of the thermocline or the epilimnetic-metalimnetic boundary, a determination that requires a temperature meter and some fairly sophisticated understanding of thermal structure.

The euphotic depth could be estimated easily, but not necessarily accurately, using some multiple of the Secchi depth. There are two considerations about the use of the euphotic depth as the lower limit. First, euphotic depth (1 percent of surface light) is not a fixed multiple of Secchi depth. Although the computation of 2X Secchi depth is often used, the Secchi depth is not a constant function of sub-surface light intensity as is euphotic depth. Estimates of euphotic depth have been reported to be from 1.16 to 2.3 times the Secchi depth (Davies-Colley and Vant 1988). Because this multiple varies as a function of a number of uncontrollable environmental variables, the euphotic depth, as estimated by Secchi depth, is a very rough estimate indeed.

The second consideration about using euphotic

depth is assessing the need for obtaining concentrations in the euphotic zone in the first place. Euphotic depth is considered to be the maximum depth where photosynthesis occurs. Knowledge of the concentration of chlorophyll in the zone of photosynthesis is important if photosynthesis is being modeled or estimated. However, if the intent of gathering data is to estimate the total (or average) concentration of chlorophyll in the lake, not just the euphotic zone needs to be measured. The concentration of phosphorus or any material other than chlorophyll in the euphotic zone has no intrinsic meaning, other than if a relationship exists with chlorophyll. It is possible that, in different lakes, the euphotic zone may be less than a meter in one and could extend into the hypolimnion in another.

The tube sampler could be lowered to a fixed depth. The same fixed depth could be used in every lake, or this depth could reflect an estimate of the mean depth of the lake, the epilimnion, or the thermocline in each lake. The coordinator could visit each lake and determine the depth of the epilimnion, but most programs are initiated in the spring, perhaps before a thermal gradient has set up. Alternatively, the thermocline or epilimnetic depth could be estimated using any of numerous equations relating morphometric measures to these variables.

Hanna (1990) devised equations relating thermocline depth to MEL, lake area, and shoreline length (Table 6.2), variables that could be obtained from a map before the sampling season began. Maximum effective length (MEL) is defined by the straight line connecting the two most distant points on the shoreline over which wind and waves may act without interruptions from land or islands (Håkanson 1981; Hanna 1990). These values could then be field-evaluated later in the season. The mean depth, if known, could also be used as the fixed depth.

Another disadvantage of the hose sampler is that it samples each depth equally, even though each depth contributes unequally to the total volume of water in the lake. The average concentration in the lake (C_{avg}) is calculated as:

$$C_{avg} = \sum_{z=0}^{z_{max}} C_{(zn\text{-}zm)} V_{(zn\text{-}zm)} \qquad (6.1)$$

$C_{(zn\text{-}zm)}$ is the concentration between depths z_n and z_m and $V_{(zn\text{-}zm)}$ is the volume of water in the same interval. The bulk of the water is at the surface, therefore, the concentration of a substance at the surface usually contributes most to the average concentration in the lake. The volume of water in the metalimnion and hypolimnion is much less, and concentrations in this region should contribute less to the average concentration.

However, when a tube sampler is used, the concentrations at each depth are weighted equally rather than in proportion to the contribution of each volume to the total volume of the lake. This equal sampling falsely increases the influence of the deeper concentrations on the average concentration. If concentrations at these lower depths are much higher than at the surface, their contribution to the lake's average concentration is higher as well. Large metalimnetic chlorophyll peaks or hypolimnetic phosphorus concentrations would have an exaggerated impact on the estimate of the average concentration. There is no guarantee that a hose sampler lowered to the depth of the epilimnion or thermocline increases the accuracy of an estimate of epilimnetic or lake concentration. Increased accuracy will depend on the morphometry of the lake and the nature of the vertical distribution of the variable concentrations.

If a program does not have the funds to sample each depth independently, yet considers that the hose sampler inaccurately measures the average concentration, an alternative would be to have the volunteer produce a composite sample. This would entail having the volunteer pour a volume of sample proportional to the volume of water at each depth into a sample container. The resulting concentration in the sample container should be very close to the average lake concentration. However, the volume of each stratum would have to be known, and the volunteer would have to carefully measure volumes appropriate for each depth. This may be more than can be expected from the average volunteer.

Several studies have evaluated the effect of vertical sampling strategies on estimates of lake concentrations. Hanna and Peters (1991) compared several strategies, including weighted-composite samples to the "trophogenic zone" (zone of water where primary productivity is greater than respiration with a positive increase in algal biomass - estimated as 2X Secchi depth), euphotic (1% light), and maximum depth, hose samples to the epilimnetic and trophogenic depths, and subsurface samples. They found that subsurface

Table 6.2.—Models that could be used to estimate thermocline depth (THER) based on maximum effective width (MEL), area (A), and shoreline length (L).

Equation	r	Sources
Log THER = 0.336 Log (MEL) - 0.245	0.92	Hanna 1990
Log THER = 0.185 Log (A) + 0.842	0.91	Hanna 1990
Log THER = 0.282 Log (L) - 0.225	0.91	Hanna 1990
Log THER = 0.443 Log (A) + 0.553	0.91	Osgood 1987

samples had the lowest phosphorus concentrations, epilimnetic hose samples somewhat higher, samples of the trophogenic and euphotic (1% light) still more, and water column composites (total water column) the highest. In their lakes the maximum difference of the means was only slightly more than 20%.

Chlorophyll concentrations were also smallest in the subsurface samples, higher in the epilimnetic hoses, higher yet in the volume-weighted samples, and highest in the hose samples of the trophogenic zone. Although there were differences between techniques, the inter-lake differences were even greater, meaning that any of the techniques could distinguish between lakes. However, they thought that subsurface samples yielded low values and were subject to greater daily variation and therefore should be avoided in inter-lake surveys. They recommended the use of the hose sample over integrated composite samples because the hose sample requires less effort. They recommended using 2X Secchi depth because such samples represent lake productivity better in principle, although they could not demonstrate this advantage in practice.

Knowlton and Jones (1989) compared subsurface (10 cm) samples with samples continuously drawn with a pump from the surface to the oxycline, or, when no oxycline was present, from the surface through the entire mixed layer. Differences between surface and composite samples in chlorophyll and total phosphorus were usually small, but differences increased when phytoplankton formed subsurface layers. Chlorophyll concentrations tended to increase with depth, seldom being maximal at the surface. However, they considered the error obtained from differences between the two sampling techniques to be much less than temporal variation and the sampling technique would add negligible error in inter-lake surveys.

The difference between this study and that of Hanna and Peters (1991) may be the stability of the algal layers. Knowlton and Jones found subsurface chlorophyll layers to be the major cause of differences between the techniques, but these layers were transient, common only on calm days. Metalimnetic maxima were also transient, occurring infrequently in the lakes. More importantly, the concentrations in the peaks were less than 20% higher than surrounding layers. It may be that metalimnetic peaks accounted for some of the differences in concentration found by Hanna and Peters (1991). In their lakes the peaks may have been more prevalent and more stable. This would explain why hose samples actually had higher chlorophyll concentrations than did weighted composite samples.

The final word on the best method to sample has yet to be written. Surface samples are the easiest to measure and require the least equipment. However, they may be more variable and under-represent the concentrations in the lake. Hose samplers are marginally more expensive and may sample more of the water column, thus reducing variability caused by vertical heterogeneity. However, if high concentrations are found deeper in the sampled water column, the hose sampler may over-represent the epilimnetic or lake concentration. Also, there is no agreement as to the appropriate depth the hose should be lowered. A volume-weighted composite sample may be best, but it requires the knowledge of the lake volume at each stratum and more effort on the part of the volunteer.

When To Sample

Deciding on when, how long, and how frequently it is necessary for the volunteers to take samples is as fraught with statistical decisions as for sampling location and depth. There seems to be no statistically acceptable method that coincides well with the realities of using volunteers to collect samples. Because of this, some compromises may have to be made. These are discussed below.

The length of the season that the volunteers are asked to sample is dependent on the goals of the program. If, for example, the goal is to obtain annual mean concentrations or to examine the annual fluctuations in the target variables, then the calendar year is the appropriate duration of the sampling season. If ice-free or summer mean concentrations are desired, then the sampling period becomes obvious.

If a whole-lake springtime phosphorus concentration is needed for a nutrient budget or to calibrate a loading model, then sampling should include the period of spring turnover. If that spring turnover total phosphorus concentration were used in a model to predict summer chlorophyll (Dillon and Rigler 1973), then calibrating that model would require summer chlorophyll concentrations as well.

It may be a little more difficult to select a sampling season if the goals are to determine concentrations or parameters that may not be dependent on a certain season, i.e. maximum concentrations. Peters (1990) points out that the typical limnological preoccupation with the summer season may miss spring and fall peak chlorophyll concentrations, which, according to Marshall and Peters (1989) may be higher than peak concentrations found in the summer, especially in eutrophic lakes. Marshall and Peters (1989) point out that most models predict mean chlorophyll concentrations and ignore the temporal distribution of chlorophyll.

The result is that when such models are used, the resulting predictions may be irrelevant to the program goals. Does the program need to know the annual or seasonal mean of chlorophyll or phosphorus, or is the peak concentration of more interest? Will a single number (the mean) be all that is used of the volunteer

data, or are seasonal fluctuations of importance as well? Does it matter that the maximum comes in April or is the peak concentration in July or August of more interest?

No single recommendation can be made regarding sampling duration. The duration depends on the goals of the program. The better these goals are defined, the easier it will be for the coordinator to choose a sampling period. Once this is done, the reality of volunteer cooperation and capability should probably be considered. An annual mean requires that someone must visit the lake throughout the calendar year. Will volunteers be willing to do so? In the northern states, being on the lake in the winter or early spring or late fall can be cold and dangerous. Some volunteers might not even launch their boats or go to their cabins until May or June. The coordinator may have to modify the program expectations to incorporate the schedules of the majority of the volunteers.

The frequency of sampling is another important consideration in the design of the volunteer program. If the coordinator has the volunteers sample too infrequently, then there is a good chance that the variation will be so large that it will be impossible to statistically identify differences between lakes or between sampling years. If the volunteers sample too frequently, their enthusiasm may wane and the added statistical reliability may not be justified. Oversampling will also waste money that could have been used to fund more lake monitoring. When sampling frequency translates into dollars and volunteer time, it becomes necessary to balance the need for statistical confidence against the monetary and human costs incurred in obtaining that level of confidence.

Most volunteer programs probably have as one of their goals the estimation of the annual or seasonal statistic of central tendency (mean or median) for each of the monitored lakes. Probably this statistic will be used to compare lakes and to monitor year-to-year changes in each lake. In order to do either, a sufficient number of samples must be taken so that a reliable estimate of the true central tendency can be obtained. The term "reliable" in this case is relative; it depends on the level of uncertainty that the coordinator will accept. The less uncertainty (the more precision) desired, the more samples will have to be taken.

Two methods have been used to estimate the number of samples needed in a monitoring program; both are discussed in Marshall et al. (1988). The first, also discussed in Gaugush (1987), Reckhow and Chapra (1983), and Thornton et al. (1982), uses the equation

$$n = \frac{t^2 s^2}{d^2} \qquad (6.2)$$

Where:

- n = number of samples
- t = appropriate value from Student's t distribution for the degrees of freedom (n-1)
- s^2 = sample variance
- d = desired precision about the mean, expressed in the same values as the mean (e.g., µg/L).

The term d can also be written as $r\bar{X}$ where r represents the desired precision as expressed as a fraction and $\bar{X}$, the mean of the sample (Gaugush, 1987). For example, if the mean of the chlorophyll concentration was estimated at 20 ug/L, and the desired level of precision is ± 10 percent, d would have the value 0.2 (0.1 x 20). The equation then looks like:

$$n = \frac{t^2 s^2}{r^2 \bar{X}^2} \qquad (6.3)$$

To use this equation, the coordinator first must decide what level of precision is desired, and have some estimate of the mean and variance of the target variable. The desired precision in most experiments is commonly 5% error, but to obtain this level of precision is very difficult in limnological monitoring programs without a very large number of samples. More often, values of 10 or 20% are found to be more possible (Gaugush 1987; Marshall, et al. 1988).

There are several commonly used methods used for estimating the mean and variance of a particular variable (Gaugush 1987). First, the coordinator could conduct a pilot study, gathering sufficient data to estimate mean and variance. Second, the results of previous studies on the lake or on similar lakes can be used. The third method, according to Gaugush (1987), would be to make an educated guess. A fourth method has been reported by Marshall, et al. (1988), for chlorophyll and by France and Peters (1992) for total phosphorus. This method relies on the reported relationship between the log variance of an annual or seasonal sample and the log mean value of the same sample (Walker 1985). Marshall, et al. (1988) report the relationship between variance (s^2) and mean for chlorophyll to be:

$$\log(s^2) = 0.53 + 2.10 \log\bar{X} \qquad (6.4)$$

for annual data, and

$$\log(s^2) = -1.03 + 2.50 \log\bar{X} \qquad (6.5)$$

for seasonal data. France and Peters (1992) report the relationship for total phosphorus to be:

$$\log(s^2) = -0.563 + 1.65 \log\bar{X} \qquad (6.6)$$

for seasonal data (April - October).

Marshall, et al. (1988) used these values in Equation (6.2) above to obtain estimates of the variance based

solely on the mean value. Knowledge of the mean is still necessary, but again it can be estimated from similar lakes or previous studies, or from information on Secchi depth that can be related to total phosphorus or chlorophyll.

The most difficult part of estimating n is the determination of t, the value from the Student's t distribution for the degrees of freedom (n-1). This value depends on a knowledge of n, the value you are seeking. Therefore an iterative approach is required, where an initial guess of the value of n is used to obtain t, and then this calculated value of n is used to estimate t, and so on until n converges on the best estimate of n (Thornton, et al. 1982; Gaugush 1987).

Marshall, et al. (1988) estimates that 10 observations per year would be needed to estimate the seasonal mean of chlorophyll with a coefficient of variation of 20 percent. Between 30 and 40 observations would be needed to obtain a coefficient of variation of 10 percent. Total phosphorus has a lower variance around the mean than does chlorophyll, and fewer samples would be needed (Knowlton, et al. 1984; France and Peters 1992). However, if a relationship between chlorophyll and phosphorus is desired, then the larger number of samples might be desirable for each. Since the variance apparently increases as the mean of the sample increases, oligotrophic lakes would not need as many samples taken as would a eutrophic lake (Marshall, et al. 1988).

Temporal Sampling Strategies

Sampling the lake over the year could be done by randomly pre-selecting sampling dates for the volunteers. Having the volunteers sample the entire calendar year may have some virtues, but the needs of most programs, at least in the northern states, might consider using the ice-free period as more important and logistically possible. Within the summer or ice-free period, there is rarely a random assortment of possible concentrations and conditions. Day length, temperature, and, in some lakes, algal chlorophyll increase to a maximum and then decrease. Inputs of phosphorus may be highest during spring runoff and these may translate into higher lake concentrations in the spring. On the other hand, internal loading in some lakes may cause a continual increase in nutrient concentrations throughout the summer.

Because of these non-random fluctuations in limnologically-interesting variables, a simple random selection of sampling dates during the summer may not adequately sample these systematic fluctuations. It may be more appropriate to stratify the sampling dates or to use a sequential sampling technique. Stratification could be done within months (June, July, etc.), within limnological periods (ice out to stratification, stratification to turnover, turnover to ice-over) or hydrologic periods (high flows in the spring, summer, low flow inputs in the summer, autumnal high flows). In any of these cases, the coordinator would supply the volunteer with a list of randomly selected sampling dates within each period, and the volunteer could be expected to sample on that date, rain or shine.

If a sequential sampling technique were used, the coordinator would first decide on the period of interest and on an appropriate time segment (7 days, 14 days, 30 days, etc.) with which to divide that period. An initial sampling day would be chosen randomly from the first time segment. Sampling would be initiated on that randomly chosen date and at equal time segments thereafter. For example, a coordinator might choose to sample every 15 days from May 1 to September 30. To do so, a date would be chosen at random between May 1 and May 15 (a 15 day interval). If May 6 were chosen, sampling would start on May 6 and then at 15 day intervals (May 21, June 5, etc.) throughout the summer. New starting dates would be chosen for each lake or volunteer.

A sequential sampling program might seem the easiest to organize because the volunteer would know when they sample, but since the initial sampling times are randomly predetermined, it might mean that the volunteer would have to sample every Thursday, even though they go to the lake only on weekends. More importantly, as mentioned earlier, it is difficult to obtain an accurate measure of the standard error of the mean from a systematic sampling effort. In this case, even a rigid sampling regime may produce statistical problems in interpretation.

Although a random or a sequential design could be used for a volunteer program, the reality may be that it will be difficult to get volunteers to agree to go out to sample on the assigned days. On some lakes it may be dangerous to go out on days when the boat traffic is highest. Certainly no volunteer should go out if there is a threat of lightening or high winds. Rainstorms may discourage others. Volunteers may only go to their cabins on weekends, and sampling would therefore be limited to certain dates. Any of these instances would violate one or more assumptions of the sampling regime, and therefore increase the possibility of biased estimates.

It is the question of bias that should be of concern. The goal of the program should be to obtain the least biased estimates of the condition of the lake. A sampling date chosen by the volunteers could introduce bias. For example, if a lake is only sampled on a weekend, boat traffic may temporarily increase turbidity, and therefore lower Secchi depths would be recorded. On the other hand, increased numbers of visitors at the lake during the weekend might increase nutrient inputs from septic tanks, which, in turn, might

translate into higher algal densities during the next week.

In the first instance sampling, for example, every Wednesday would have a larger Secchi depth than sampling on Sunday; in the other, it might be smaller. Consider the validity of comparing the chlorophyll concentrations in two lakes, one sampled on Sunday, the other on Wednesday. Without evidence that there are no regular weekly fluctuations, the comparison could be invalid. In some lakes, sampling during or after a rainstorm may produce Secchi depths and nutrient concentrations altered by inputs of silt and clay from runoff. Three days later, the lake may be clear or it may have higher algal populations. Statistically valid sampling techniques assume that each of the events has a equal chance of being sampled, and each event contributes equally to the final estimate of central tendency.

A range of temporal sampling strategies seems possible. At one extreme, the coordinator provides a rigid sampling scheme that is statistically correct, either using a random, a stratified random, or a sequential sampling design. Volunteers would be required to adhere to the sampling regime to be a part of the program. Although it might sound rigid, it would provide the needed statistical rigor.

The other extreme would be to ignore the whole business of sampling strategy, hoping that there is sufficient randomness and minimum bias in the volunteer sampling that the numbers will still approximate the population mean. For example, volunteers could be urged to sample twice a month, suggesting that the two dates not be close together. No more guidance would given as to possible sampling dates, relying on the judgement of the volunteer.

Between these two extremes the coordinator could establish a sampling "concept," in which volunteers attempt to sample within certain time periods. For example, volunteers could be asked to sample within the 2 week period, July 1 to 15, plus or minus a given number of days (June 1 ± 1 week).

The central day or the time period would be chosen at random by the coordinator. The actual sampling date would not be randomly selected, but selected at the convenience of the volunteer. This scheme would provide some random structure to the sampling regime, but allow some flexibility to the volunteer.

It should be obvious that the first extreme may be too rigid for a volunteer program and the other too haphazard. The third still may contain the weekly biases mentioned above. If, however, an effort was made to determine if there were or were not weekly sampling biases, the procedure might have more validity. It could be suggested to the volunteers that they measure Secchi depth several times in a single week. From these multiple measurements, some estimate of within-week variability might be obtained. If there was a strong weekly pattern, then some accommodation may have to be made. If not, then there would be some confidence that the sampling "concept" may also be statistically valid.

In the case of sampling during or after rainstorms, it may be that it would be easier to filter the data after collection rather than to urge the volunteer to endanger themselves adhering to a rigid schedule. Volunteers could be urged to write the weather conditions for the past 3 days on their note card. When the data are analyzed, the coordinator could test as to whether prior precipitation had an effect on the Secchi depth or any other variable. Then, if necessary, the coordinator could eliminate all data in which precipitation occurred within a 24, 48, or 72 hour period prior to sampling.

Volunteer Lake Monitoring Programs in the Upper Midwest

Illinois Volunteer Lake Monitoring Program

Sampling Location

Three monitoring stations are established on most lakes. One station is over the deepest portion of the lake. (Since most of the lakes are impoundments, this station is usually established near a dam.) The second site is located at mid-lake or in a major arm. The third site is near the headwaters. The sites are marked on a map and volunteers are trained to locate the sites using a technique called "triangulation."

Basically, the triangulation technique involves making the initial visit to the site and noting two sets of permanent objects on the shore that line up one behind the other. The two sets of lined-up objects plus the designated site location form the triangle (Fig. 6.2). On subsequent visits to the site, the volunteer knows he/she has found the proper location when the objects again form the visual triangle. In Illinois, volunteers are instructed to further verify they are in the correct location by taking a depth measurement as well.

Secchi Disk

Volunteers in the Illinois Program are instructed to take Secchi disk readings at least twice a month, once between the first and fifteenth and once between the sixteenth and the end of each month. Officials request that volunteers take their readings as close to midday as possible, or at least try to schedule the trips at the same hour of the day between 10:00 a.m. and 4:00 p.m. The sampling season runs from May through October.

Water Sample Collection

Volunteers in the advanced monitoring component of the Illinois Program are trained to collect water

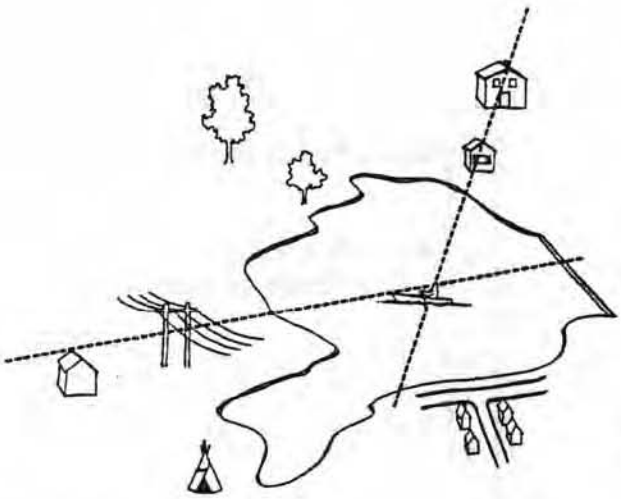

Figure 6.2—Diagram of the triangulation method (from the Standard Operating Procedures document of the Ohio Citizen Lake Improvement Program)

samples once a month for laboratory analysis of total and volatile suspended solids, ammonia nitrogen, nitrate + nitrite nitrogen, and total phosphorus. Samples are to be taken at a depth of one foot at each of their assigned sites. Samples are stored in two sample bottles and shipped to the Illinois EPA laboratory for analysis.

The sampling equipment provided to the volunteer includes:

- Quart bottle (for the suspended solid analysis);
- 4 oz. bottle with nitric acid (for phosphorus and nitrogen analysis); and
- Insulated shipping box with 48-hour ice packs.

Listed below are instructions for the collection of the surface samples. They are generalized from the sampling protocol sheet given to Illinois volunteers.

- Using a waterproof marker, label the sample bottles provided by the Illinois EPA laboratory with the lake name, site number, date and time.
- Upon reaching the lake site, rinse out the quart bottle by immersing it with the cap on, neck first, into the water to a depth of one foot.
- Remove the cap underwater, keep your hand away from the bottle opening, and allow the bottle to completely fill by raising the neck above the rest of the bottle.
- Replace the cap underwater, raise the bottle out of the water, and discard the water on the opposite side of the boat.
- Refill the quart bottle in the same manner, cap it underwater and raise it out of the water.
- Fill the 4 oz. bottle to the neck and cap it, being careful not to overfill the bottle. This must be done because the small bottle contains a preservative acid which may be diluted or washed out of the bottle if it is immersed in the lake.
- Discard the remaining water in the quart bottle
- Refill and cap the quart bottle in the manner described above.
- Immediately place both bottles into a cooler with ice.

The volunteer is to refrigerate the samples and ship them to the state laboratory as soon as possible (within 24 hours).

Indiana Volunteer Lake Monitoring Program

Sampling Location

Volunteers are instructed to establish a site over the middle (deepest) part of the lake.

Secchi Disk

Secchi disk measurements are to be conducted at least once every two weeks from May through October. Weekly sampling is encouraged if the volunteer has time, but they are asked to wait at least five days between readings. Volunteers are instructed to take readings between 10:00 a.m. and 4:00 p.m.

Water Sample Collection

Volunteers participating in the advanced portion of the Indiana Volunteer Lake Monitoring Program are trained to collect an integrated sample of water once a month using a hose. The depth range sampled is from the surface to six feet deep. The sample is transferred from the hose to a pitcher container.

A portion of the sample water is poured into a 125 milliliter clear bottle that has been acid-washed. This water is analyzed in the laboratory for total phosphorus concentration. A portion of the remaining water in the pitcher is filtered for laboratory analysis of chlorophyll *a*. Analysis takes place in the Clean Lakes Laboratory, School of Public and Environmental Affairs, Indiana University.

The water sampling protocol used by the Indiana program was adapted from Vermont's citizen lake monitoring program. Listed below are the instructions for the collection of the integrated sample generalized from a training document given to Indiana volunteers.

- Rinse the pitcher (used to hold the sample) with lake water twice by simply dipping the pitcher into the lake.
- Rinse the hose by slowly lowering the weighted end of the hose into the water so that the six foot mark on the hose is two feet below the surface.
- Without using the rope, slowly pull the sampling hose back up and into the boat, then repeat.
- To take the integrated water sample, slowly and evenly lower the weighted end of the hose into the water until the six foot mark on the

hose is even with the surface of the water.

- Fold the hose twice just above the surface of the water (above the six foot mark) and hold the crimp firmly in your hand. Always hold the crimp higher that the rest of the hose to prevent backwashing into the top end of the hose.
- Lift the weighted end out of the water with the rope while keeping the crimped top end above your head.
- Hold the weighted end of the hose over the pitcher. In order to prevent contamination, be careful not to let the coupling on the weighted end of the hose touch anything (your hands, the pitcher, the water that you will empty into the pitcher).
- Is the crimped end of the hose higher than the weighted end? If so, then slowly release the crimp in the hose.
- Proceed to move the hand that was holding the crimp towards the weighted end of the hose. Remember the weighted end of the hose must always be lower than the top end of the hose.
- The water in the hose should pass out of the weighted end of the hose and into the pitcher. Repeat the procedure until you have the correct amount of water in the pitcher.

It is noteworthy that in 1992 two Indiana program volunteers independently designed integrated pipe samplers after struggling with the hose sampler described above. Fig. 6.3 is a sketch of one of the designs. This feedback from these and other volunteers has resulted in a switch in sample collection protocol. Currently all new expanded program volunteers receive, and old volunteers are encouraged to make, this simple 1 inch diameter PVC water pipe integrated sampler with a ball valve on the bottom. This input further illustrates the level of commitment of volunteers and how they contribute to the success of monitoring programs.

Both the phosphorus and chlorophyll *a* samples are frozen and shipped next-day or Priority Mail after two samples have been collected.

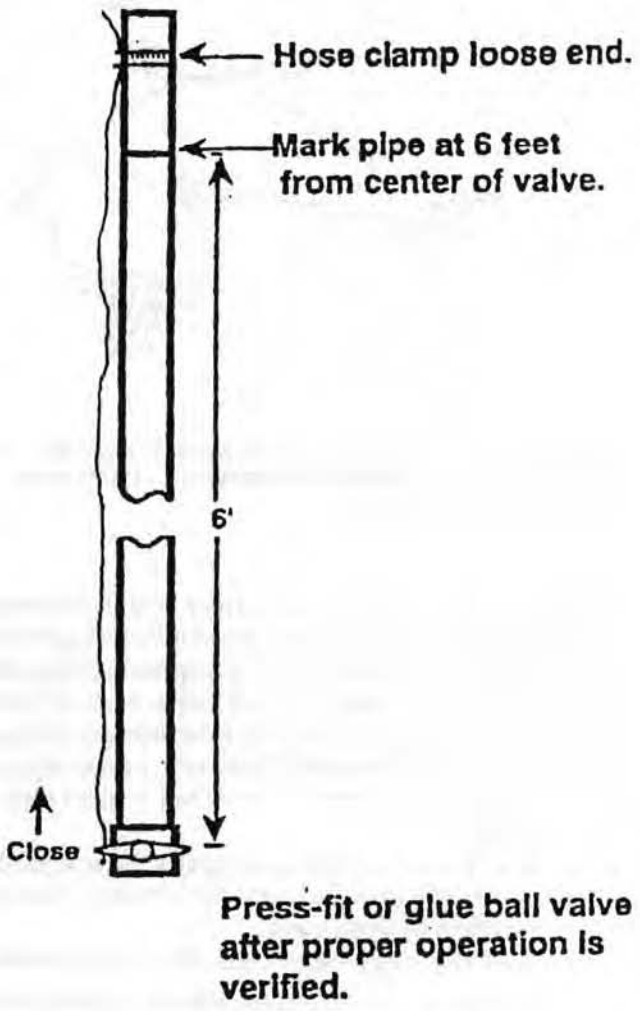

Figure 6.3—Diagram of the Indiana program's water pipe integrated sampler

Michigan Inland Lake Self-Help Program

Sampling Location

Volunteers are requested to establish a monitoring site over the deepest part of their lake. In a few lakes measurements are taken in two or three distinct basins.

Secchi Disk

Volunteers are instructed to monitor Secchi disk depth once a week from mid-May through early September. Readings are to take place between 10:00 a.m. and 3:00 p.m.

Water Sample Collection

Volunteers in the advanced monitoring component of Michigan's program collect surface grab samples for total phosphorus approximately two weeks after ice out. Volunteers are given written instructions and trained to collect the sample the sample to minimize sample contamination. Samples are collected in duplicate and frozen immediately after collection. Samples are delivered to and mailed from a local MDNR office to the State's laboratory in the frozen state.

Minnesota Citizen Lake-Monitoring Program

Sampling Location

The volunteer is instructed to "select one location, well offshore and in a deep part of the lake and remain with that site throughout the sampling season," but, ultimately, the selection of the sampling location is at the discretion of the volunteer. If, however, there are distinct bays in the lake, volunteers are encouraged to select multiple sites. All sampling sites are marked on a lake map with the volunteer retaining a copy of his/her map and the MPCA maintaining a master map file of all lakes monitored that shows all sites ever monitored.

Secchi Disk

Volunteers are requested to take Secchi disk measurements once a week, but to space them at least three days apart. The program normally begins in mid-June and ends in September. Volunteers are requested to take their readings between 10:00 a.m. and 3:00 p.m.

Water Sample Collection

There is no water sampling component in the Minnesota Citizen Lake-Monitoring Program.

Ohio Citizen Lake Improvement Program

Sampling Location

Ohio volunteers are instructed to establish a sampling station over the deepest part their lake. They are trained in the triangulation method of finding their sampling site.

Secchi Disk

Volunteers collect Secchi disk data during the first and third weeks of the month. They are requested to try and allow a two week period, or at least ten days, between monitoring trips. This period of time, officials explain, is needed in order to get an overall view of conditions during the month. Officials request that volunteers monitor between 10:00 a.m. and 4:00 p.m.

Water Sample Collection

There is no water sampling component in the Ohio Citizen Lake Improvement Program.

Wisconsin Self-Help Lake Monitoring Program

Sampling Location

Wisconsin volunteers and WDNR Lake Specialists together establish a sampling site over the deepest portion of the lake. If a lake has more than one distinct basin, multiple sites may be designated. Volunteers are trained in the triangulation method to find their sampling site.

Secchi Disk

Volunteers measure Secchi disk depth at least once every two weeks. Weekly readings are encouraged, but they are asked to wait at least five days between measurements. Monitoring typically begins in April so records will include spring overturn data. Many volunteers continue their season to lake freeze. Readings are conducted between 10:00 a.m. and 4:00 p.m.

Water Sample Collection

Volunteers in the advanced monitoring component of the Wisconsin Program are trained to collect point samples in the water column with a horizontal point water sampler. Water temperature and dissolved oxygen concentration are measured at specific intervals (usually three feet). Temperatures are read by the volunteer immediately after the sample is taken. Dissolved oxygen concentrations are determined by the volunteer on shore using a portable analysis kit. In addition, the volunteer collects a water sample at a depth of three feet. On shore the volunteer filters a specific volume of this sample through a filtering apparatus for chlorophyll *a* analysis in the laboratory. Water samples are also collected at three foot depth and three feet off the bottom for analysis of total phosphorus concentration. Laboratory analysis is conducted at the Wisconsin State Laboratory of Hygiene.

The equipment provided to the volunteer for the collection of water samples includes:

- Two 250 ml plastic bottles;
- A two-quart plastic juice jug;
- Dissolved oxygen bottles (if they are participating in the Expanded Program); and
- A horizontal point sampler (with a thermometer mounted internally).

Listed below are instructions given to volunteers regarding use of the sampler generalized from the training manual provided to Wisconsin volunteers.

- Hold the water sampler horizontally. Using the plastic rods, pull the sealing balls from both ends of the sampler and bring the metal rings together. Hold rings together firmly with one hand. Attach and close the metal snap shackle. Position the snap shackle split ring away from the bottle to assure proper release. Note: Twist on the sealing ball to adjust the position of the shackle.
- Make sure the hose clamp is pinched closed. Lower the sampler to the desired depth using the one-foot marks on the lowering rope for reference. Once the sample is at the appropriate depth, give the rope a short, sharp tug to release the sealing balls. (It may be helpful to practice several times on land to get a feel for how the sampler works.)

- Bring the sampler to the surface. Place the rubber spout in the desired sample bottle. Release the hose clamp by pressing the top lip forward. Release the vacuum by cracking the top ball seal to release the water.

Listed below are the specific instructions for collecting the water sample for phosphorus analysis.

- Prepare the water sampler and collect a water sample from 1 foot below the surface.
- Pour water from the hose into a 250 ml bottle. Cap the bottle and mark both the cap and bottle "3 foot" using the waterproof pen.
- Place the sample out of the direct sunlight until you return to shore.
- Prepare the sampler again and collect a water sample from approximately 3 feet from the lake bottom. Be careful not to hit bottom with the sampler.
- Pour water from the hose into the other 250 ml bottle. Cap the bottle and mark both the cap and bottle with the exact depth from the surface from which the sample was taken.
- Place the sample out of the direct sunlight until you return to shore.

The Wisconsin training manual discusses the importance of not disturbing the sediments when collecting the sample two feet off the bottom. It informs the volunteers that the sediment is often rich in nutrients from the accumulation of organic matter. Therefore, stirring them up with the sampler will yield a sample that is unrepresentative of the bottom water. The volunteer is instructed to retake the near-bottom sample from a slightly different position if he/she observes any sediment in the sampler.

As mentioned above, the Wisconsin volunteer also collects a water sample for chlorophyll *a* analysis. Listed below are the specific instructions for collecting this sample.

- Take the 2-liter plastic jug out of your sampling kit. Remove the lid and place the jug at your feet.
- Prepare the water sampler and collect a water sample from a 3-foot depth.
- Pour water from the sampler into the plastic jug.
- Repeat the steps in order to fill the jug to the top.
- Cap the jug and store out of direct sunlight.

Recommendations

This chapter has attempted to deal with the problems of obtaining statistically reliable data from a volunteer program. In a real sense, the problems discussed in the chapter are not limited to volunteer programs, but are commonly encountered in professional limnological investigations. The solutions are varied, but too often the solution is to ignore rather than to deal with the problems. Limnologists should be a great deal more uncomfortable about the pronouncements they make about the limnological conditions of a lake based on a single sample taken once weekly at one site from May until September.

Using volunteers instead of trained professionals only adds to the problem because it is more difficult to control when, where, or how samples are taken. The coordinator must rely on non-professional eyes to avoid non-normal situations and on non-professional integrity that shortcuts in technique, location, or sampling dates are not used. The volunteer is being asked to shoulder a considerable burden, and may not fully realize the importance of that burden.

The solutions offered in this chapter often center around two themes. The first theme centers on the fact that statistical validity is important and the realities of volunteer sampling must be recognized within that context. If the coordinator and the volunteer both realize that it is important to sample on certain days, at certain sites using careful, repeatable, defined procedures, then a first step has been made towards statistical validity of the data. The second step is made when the coordinator recognizes, and deals with, the known or potential biases included in the sampling procedures used by the volunteers.

The second theme reduces to one of acknowledging the potential biases remaining in procedures. In the same sense that one would not make pronouncements about the annual mean concentration of phosphorus based on an intensive summer sampling procedure, one should recognize that non-randomly selected lakes do not necessarily allow unrestricted statements about the condition of the state's lakes.

Samples taken at the surface may or may not represent the average content of the lake, and samples taken at a single point do not necessarily represent the entire extent of variation within the lake. One can, however, make statements regarding the average summer concentration of phosphorus at the surface at a location near the center of the lake. If that station is well marked and could be returned to repeatedly, then weekly, monthly, or yearly fluctuations of the surface water phosphorus at that site could be measured and compared. The following recommendations are made.

1. The coordinator should clearly and completely define the goals of the monitoring program. These goals should be used to guide decisions as to sampling times, locations, etc.
2. It may be that availability of volunteers, rather than the coordinator's needs and desires, will decide the lakes that are sampled and the locations and times that samples are taken. The

coordinator should carefully consider the biases introduced by these non-random elements in the sampling program. Efforts should be made to eliminate biases, filter the data, or assure oneself that the amount of bias introduced is not significant, relative to the needs of the program.

3. At least one permanent sampling site should be chosen for each lake. This site should be over the deepest portion of the reservoir or lake, so that vertical samples can be taken of the entire water column. This site should be well-marked, so that it could be used in the future by other volunteers or professionals. This primary site may not characterize the entire lake basin, but can serve as a point of comparison from year to year.
4. Volunteers should be urged to sample at more than one site. In dendritic or low residence time reservoirs or lakes, samples should be taken at several sites. These sites should also be well-marked.
5. No recommendation is made as to how to best sample a water column. Surface samples are inexpensive but may have high variability and underestimate epilimnetic and lake concentrations. Hose samples may integrate the waters of the lake, but may cause overestimation of lake concentrations. A volume-weighted composite sample or individual samples at several depths may be the best method, but may involve too much labor and laboratory expense for volunteer programs.
6. The seasonal period of sampling may depend on the goals of the project and the latitude of the sample lakes. For many purposes, seasonal or ice-free sampling seasons is acceptable. Shorter sampling periods may reduce variability, but also reduce information on seasonal variation.
7. It is recommended that some random structure be given to deciding on sampling days, while still giving the volunteers some degree of freedom for selecting convenient days. Within-week sampling bias should be considered prior to analysis of the data and the data filtered if necessary.

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7.
Trophic State

Section 314 of the Clean Water Act requires that all lakes of the Nation be classified according to their "eutrophic" character. Federal requirements for the Section 305b of the same Act requires that "fishable" and "swimmable" goals be constructed for each state. Federal requirements such as these have resulted in the construction and use of numerous classification schemes (Brezonik and Shannon 1971; Carlson 1977; U.S.E.P.A. 1974; Vollenweider and Kerekes 1980). These indices vary considerably in approach to classification and in the variables used for classification. All of these indices are "trophic state indices." They are predicated on the ideas that: (1) the "trophic state" of a lake is an important water quality variable; and (2) indices can determine not only the trophic character of the water body, but also if it is usable for fishing or swimming.

If trophic state classification is as valuable as the interest in it suggests, then it would be advantageous if volunteers could aid in the collection of classification data. Often, however, people perceive the trophic state concept and the ensuing classification systems as too complex for volunteers. This is simply not true. There are simple approaches to trophic state classification that are not only easy to use, but actually closer to the original intent of the concept. The purpose of this chapter is to give some perspective and guidance to the coordinator with regard to trophic classification systems and to suggest how they can be used in volunteer programs.

To understand the complexity and confusion associated with the present concept of trophic state, it is necessary to begin with a brief excursion into the history of "trophic state." We'll tease apart some of the numerous strands that are woven together into the present concept, and suggest that some of the strands are not as important to the concept as others, and therefore, can and should be discarded.

Trophic State—The Concept

Einar Naumann, a Swedish limnologist at the University of Lund, Sweden, first developed what we now think of as the trophic state concept in the early part of this century (Naumann 1919). Although trophic state is usually associated with his classification system, it is important to realize that Naumann saw the classification as an artificial outgrowth of a biological reality. From a 1929 paper, Naumann's concept of trophic state can be summarized with four clear propositions about the relationship of the watershed to the functioning of a lake.

1. The amount of algae (production) in a lake is determined by several factors, primarily by the concentration of nitrogen and phosphorus.
2. Regional variations in algal production correlate with the geological structure of the watershed; lakes in agricultural, calcareous regions were greener than lakes in forested, granitic watersheds.
3. The amount of production in a lake affects the lake biology as a whole.
4. There are certain evolutionary (ontological) connections between lakes of the various types; lakes become more productive as they age.

The trophic state concept, as described by Naumann, began with the watershed. Nutrients and other chemicals from the watershed, together with factors such as temperature and light, affected the abundance of algae (production) in the lake. Production, in turn, affected the biological structure of a lake. A moment's reflection makes us realize that this is a very contemporary interpretation of the matrix of factors affecting a lake's biology.

These statements are neither some fuzzy speculation nor rigid dogmatism about how lakes should be classified; instead, they are specific, testable statements about connection between watershed characteristics and the biology and the ontogeny of lakes. In Naumann's statements are the beginnings of ideas of nutrient loading, biomass-phosphorus relationships, and potential changes in trophic status as a lake ages.

What is also remarkable about Naumann's ideas at this time is his insistence that there should be a regional approach to the study of limnology. He wrote in 1929:

> *"The advancement of the science of water-types — and of regional limnology as a whole — is of course dependent upon the collection and comparison of as abundant data as possible from different countries...In this respect our special journals could greatly further the advance of limnology by making it an absolute condition for publication that contributions should provide the data in question — without which, indeed, most such communications are quite worthless for comparative purposes."*

In the sixty years after Naumann, limnologists retreated from comparing lakes on a regional basis. It might be well for coordinators of volunteer programs to consider that they are the hope for regional limnology that Naumann envisioned so many years ago.

Trophic State—The Classification System

Einar Naumann also developed what we now think of as trophic state terminology, using terms that Weber (1907, as cited in Hutchinson 1969) used to classify the nutrient content of bogs. According to Weber, *oligotrophic* bogs were raised bogs where the nutrients had leached out, while *eutrophic* bogs were sunken, and nutrients accumulated in them. Thus the idea that oligotrophic means "poorly-fed" and eutrophic means "well-fed" originated from the nutrient condition of bogs, not lakes (Hutchinson 1969).

Naumann used the terms, but not necessarily Weber's concept, in classifying lakes. He based his original trophic classification on the "quantitative production of phytoplankton" (Naumann 1929). Oligotrophic lakes were those with low production, "never leading to a coloring or even a clouding of the water." In eutrophic lakes, production attains very high values, "the water being, for the most part, very strongly clouded or even completely colored."

Naumann related these trophic lake types to the physical and chemical factors that affect production. These factors included temperature (with which he divided the world into Arctic and Alpine, Temperate, and Tropical zones), light, and chemical factors (calcium, humic content, nitrogen and phosphorus, iron, pH, oxygen, and carbon dioxide). He divided the possible range of values for each of these factors, which he called milieu-spectra, into low (*oligotypus*), medium (*mesotypus*) and high (*polytpus*) "size-classes" or groups. For example, an oligotrophic lake might have oligotypus values of N and P and oligo or mesotypus levels of humus (Naumann 1929). Each of Naumann's original lake types may have been based on a measure of production, but he combined the measure of production with a description of the factor (or factors) that were related to that production. However, he emphasized that trophic classification was based on production, not the factor determining that production. He considered nitrogen and phosphorus to be the primary determinants of production.

As more lakes were studied, it became evident that, although some of these lakes had production as low as in oligotrophic lakes, the biological community was distinctly different from the typical oligotrophic lake. Often these lakes were at the extremes of chemical axes other than the nutrient axis, and therefore considered to be new types when production was largely affected by factors other than nutrients. The *dystrophic* lake type, actually described by Thienemann (1921), had low N and P, but moderate to high content of humus material. *Argillotrophic* lakes had low productivity but the primary trophic factor was the abundance of clay in the water. *Acidotrophic* lakes, found at pH values less than 5.5, had as low productivity as oligotrophic lakes, but a different biological community (Naumann 1931; 1932). A list of many of Naumann's lake types are given in Table 7.1. How we might envision Naumann's concept of the relationship between production and the trophic types is illustrated in Fig. 7.1.

In Germany, August Thienemann simultaneously developed a classification scheme based on the species of benthic organisms in lakes and the importance of the hypolimnetic oxygen concentration on their species composition (Thienemann 1921). It must have seemed reasonable at the time that these two classifications could be joined, because Naumann's eutrophic lakes also lacked oxygen in the hypolimnion and had distinct benthic fauna (Thienemann 1921). For a while, the marriage of these systems seemed perfect and the study of trophic classification grew rapidly (Rodhe 1975).

Table 7.1.—A list of lake types (Naumann 1929).

Lake Type	Characteristics
Oligotrophy	Low production associated with low nitrogen and phosphorus
Eutrophy	High production, associated with high nitrogen and phosphorus
Acidotrophy	Low production, associated with low nitrogen and phosphorus, but also with pH values less than 5.5
Alkalitrophy	High production, associated with high calcium concentrations
Argillotrophy	Low production, associated with high clay turbidity
Siderotrophy	Low production, associated with high iron content
Dystrophy	Low production, associated with high humic color

As often happens with classifications of Nature, lakes were found with characteristics of more than one of the established types. Hypolimnetic oxygen was supposed to be a primary discriminator between oligotrophy and eutrophy, but it was found that hypolimnetic oxygen was not solely dependent on the biological production of the lake, but was also affected by temperature and the morphometry of the basin. Tropical lakes had low algal biomass and productivity but still had anoxic hypolimnia. Lakes with small hypolimnia had anoxia despite low productivity.

Encounters with situations such as these lead to the splitting of the terminology, generating types such as morphometric oligotrophy (Lundbeck 1934, as cited in Hutchinson 1973) for deep lakes with mesotrophic to eutrophic production but, because of the large hypolimnetic volume, still had oxygen in the hypolimnion. Järnefelt (1932) proposed the term "mixotrophy" to designate lakes that had characteristics of both oligotrophy and eutrophy. Thienemann (1926) went so far as to state that the lake typology was only applicable in the temperate regions. The trophic state terminology became increasingly cumbersome as the number of recognized lake types increased. Classification became difficult, and most of the terminology finally lapsed into disuse.

Trophic State Classification in Retrospect

The seeds for the failure of the Naumann-Thienemann trophic classification were sown almost at its inception. The problems with the classification were not that a system was not needed nor that the variables chosen to classify lakes were incorrect. Instead, problems occurred because: (1) the classifications tried to incorporate all the variables, both causal and the biotic and abiotic consequences, into a single classification; and (2) people assumed that distinct sets of lakes existed which could be easily classified.

Naumann's original idea to classify lakes on the basis of production (biomass) had both practical and theoretical validity. Production was a single axis that he divided into convenient groupings (high, medium, low production) and all aquatic bodies could be classified into these groups. Naumann could map the regional variation in production and could then ask valid scientific questions as to what factor or factors might be causing observed differences in production.

Problems began when Naumann linked the production classification with the causal factors of that production. Again, a useful classification scheme could be constructed based solely on the factors that affected or limited production. Indeed, present day trophic classifications based on phosphorus are remnants of Naumann's production factor classification. The classification could have been univariate, based on the single factor that limited production (nutrients: Oligotrophy - Eutrophy, clay: Argillotrophy, humic color: Dystrophy, etc.). Instead, Naumann chose to incorporate all possible factors affecting production into the classification. He dealt with combinations of factors by combining the names. For example, an acid lake with high humic coloring and iron would be called Dys-Acido-Sidero-trophic (Naumann 1932). If he had found a lake that was high in algae as well, he might have added eutrophic to that name.

If the terminology was not cumbersome enough, production, the supposed primary standard of trophic classification, was instead the primary cause of the proliferation problem. If Naumann wanted to classify a lake on the basis of production *and* the factor associated with that production, then a new classification term had to be erected with each new potential factor affecting production. If he had known of biological growth limitations by grazing, salinity, water residence time, or morphometric factors, the classification scheme may have grown even further.

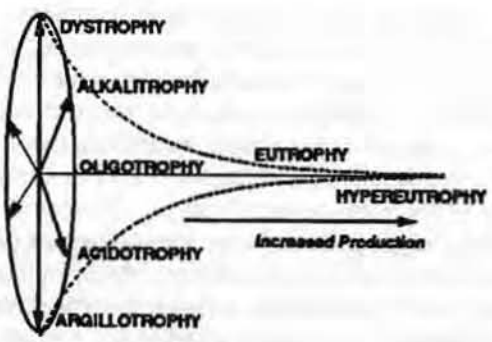


Figure 7.1—A conceptualization of the relationship between Naumann's lake types.

When biological structure, such as the benthic invertebrate community, was added to the classification scheme, the problems grew even more. Although the biological community is an important aspect of limnological ecology, it added a third level of complexity to the classification. Consequently, the scheme had to consider factors of limitation, production, and now, biological structure. However, production is only one of several factors affecting the biological community composition. Forcing biological structure into the production index denied the importance of those other factors. The result was a classification scheme that could not predict structure from production alone, yet structure was used to predict production.

A fourth level of complexity was added when hypolimnetic oxygen concentration was added to the classification. As with biological structure, hypolimnetic oxygen is also affected by factors other than production. When given equal status, the resulting classification could be affected by temperature or basin morphometry as much as by biological production.

The net result was (and is) a classification system that is "trophic" in name only. It involved the degree of production, abiotic elements that can affect that production, biological structure, and hypolimnetic oxygen concentration. Thus even in its most "developed" form, the trophic classification could only be a listing of general characteristics of generalized lakes. As a result the classification degenerated into a series of pigeon holes into which lakes were forced rather than fit.

Today trophic state terms such as oligotrophy and eutrophy are still commonly used, and terms such as dystrophy and argillotrophy can occasionally be found in the literature. Unfortunately, the original meanings of the terms have become blurred and a variety of definitions and underlying philosophies are attached to the trophic state terms. Some of these definitions bear little resemblance to the original concept.

Single Variable Indices

Some contemporary classification schemes use only a single variable to define the trophic state of lakes. This use of a single variable simplifies the classification procedure considerably because only one variable has to be measured. But which variable is the proper one on which to base the classification? Phosphorus loading, phosphorus concentration, chlorophyll concentration, algal productivity, algal biomass (often estimated via Secchi depth and / or chlorophyll concentration), and hypolimnetic oxygen deficits have all been proposed at one time or another for a single variable index. Each could be a valid candidate for trophic state classification and are briefly discussed below.

Phosphorus Loading

Vollenweider (1968a) radically changed our view of lakes when he emphasized the importance of nutrient inputs from the watershed in the determination of the concentration of nutrients and, ultimately, the density of algae in the lake. As we have seen, the idea of the impact of watershed characteristics originated with Naumann, but, only 50 years later was Vollenweider able to convince a new generation of limnologists to look to the watershed to understand the lake.

Hutchinson (1969) and Odum (1969), emphasized the importance of the watershed by defining trophic state by the loading of nutrients to the lake. For Hutchinson, trophic state was a description of the potential for a lake to respond to nutrient loading rather than a description of that response. A eutrophic system would be a system in which the total potential concentration of nutrients was high, whether or not it was expressed in a correspondingly high algal or macrophyte density. Odum (1969) and Beeton and Edmonson (1972) defined oligotrophy and eutrophy based on the amount of nutrient loading.

A series of loading graphs of Vollenweider (1968, 1975, 1976), appeared to support the idea that trophic state should be directly related to loading. Figure 7.2 illustrates how investigators with loading data are tempted to simply classify the trophic state of a lake. In this case, trophic state is determined by plotting "Average inflow concentration," which is calculated by dividing loading (L) by water loading (q_s), against hydrologic constraints (water residence time). The "Permissible" line is the boundary between oligotrophy

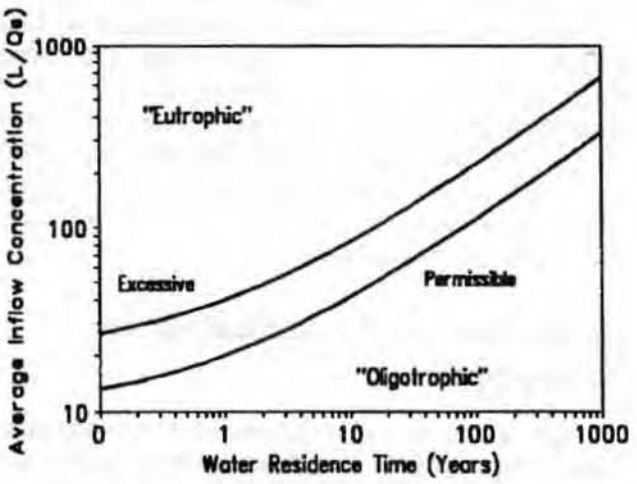


Figure 7.2—A Vollenweider loading graphic illustrating the divisions between "permissible" (oligotrophic-mesotrophic boundary) and "dangerous" or "excessive" (mesotrophic-eutrophic boundary) phosphorus concentrations.

and mesotrophy, and "Excessive" line, the boundary between mesotrophy and eutrophy (Vollenweider 1976).

In reality these lines do not represent loading classifications, but instead predict in-lake phosphorus concentrations of 10 and 20 µg/L (Vollenweider 1968, 1971, 1976). Thus, trophic state categories are actually based on the predicted phosphorus concentration in the lake, not loading. The graphs only *illustrate* the relationship between loading and predicted lake concentration. In practicality, if the investigator wanted to know the present trophic state, he/she could have simply measured phosphorus concentration, and not gone to the trouble of estimating loading. The value of the graphs and the associated models is in the *prediction* of trophic state if nutrient loading is changed, not the estimation of present trophic state.

Apart from the misuse of the Vollenweider graphs by some to estimate the trophic state of a lake, nutrient loading has not been used extensively to define trophic state. Although the impact of nutrient loading on lake conditions cannot be understated, the use of nutrient loading to define the "state" of the lake seems inappropriate. Loading can only, as Hutchinson implies, define the potential state of the lake. While potential nutrient concentration is an important measure, it does not provide the information that has attracted limnologists to trophic state classification.

Phosphorus Concentration

The concentrations of nutrients, especially phosphorus, have probably been the most popular variable for single variable trophic state indices. The dominance of trophic state designations based on phosphorus concentration rather than nitrogen is probably the result of the widespread belief that phosphorus limits algal growth in most lakes. The impact of Vollenweider's loading models on the field of predictive limnology and management also helped emphasize the role of phosphorus.

In phosphorus-limited lakes, there should be a strong relationship between phosphorus (usually measured as total phosphorus) and plant (algal) biomass. Because of this relationship, phosphorus can be used as an *estimator* or *predictor* of production. If phosphorus concentration is used to predict trophic state (biological production), nutrient concentrations are being used in the manner that Naumann originally intended.

That idea that phosphorus can predict trophic state (production) must be clearly differentiated from the use of phosphorus as the definition of trophic state. While Naumann considered phosphorus and nitrogen to be the primary factors that determined trophic state, he initially based trophic classification on the biological condition of the lake, not the cause of that condition. The trophic state of a lake is a biological condition *caused by* various factors such as nitrogen and phosphorus, but also potentially affected by pH, turbidity, color, etc.

If phosphorus is used instead of production to define the trophic state of a lake, the limnologist must be willing to forgo any accurate inference of the biological condition in a large number of lakes and reservoirs. Even the best of the chlorophyll-phosphorus models has considerable variation. More importantly, in some lakes, and certainly in many reservoirs, algal chlorophyll is not related to phosphorus concentration. In turbid lakes and reservoirs, for example, chlorophyll cannot be predicted with any accuracy because much of the phosphorus is probably attached to the non-algal particles and is unavailable for algal growth (Carlson 1992). If the biological condition of the lake is of any importance to the classification or management of a lake or reservoir, there is no justification, neither historical nor practical, to use phosphorus as the defining variable of trophic state.

Algal Productivity

Primary productivity, the rate at which light energy is incorporated into plant cells, has long been a standard of trophic state classification. Åberg and Rhode (1942) and Rhode (1969) interpreted Naumann's original trophic state definitions to be the biological productivity of a lake rather than production (the amount of plant material in the lake). Certainly, the biological dynamics of a lake and, to some extent, its biological structure, are dependent on productivity.

Although productivity is an important measure in lake evaluation, and has some historical claim as an early trophic state definition, it does have methodological drawbacks that argue against its use as the major estimator of trophic state. Traditionally, productivity requires an estimate of annual productivity, the obtaining of which requires extensive sampling.

The sensitivity of available methods for data collection is also a problem. The oxygen evolution technique is relatively easy to use and requires little equipment, but is not as sensitive as the Carbon-14 technique, which requires radioactive materials and skilled personnel. There is also the concern about radioactivity in the environment, which makes the ability to use C-14 in the field difficult if not impossible. Productivity is also subject to interpretational problems, because, when it is expressed on an areal basis ($mg/m^2/yr$), productivity can be as much influenced by non-algal attenuation of light as by the rate of plant photosynthesis. When productivity is expressed on a volumetric basis ($mg/m^3/yr$), the effective volume (epilimnetic, photic, or whole lake volume) becomes problematic (Carlson 1979).

Algal Biomass

Algal biomass is the weight of the algae in the lake and should be expressed as concentration. It can be reported as grams of dry weight, grams of carbon, or as biovolume (the total volume of plant material per volume of water). Unfortunately there is no test that can, without error, measure what we glibly call biomass. Variables such as total particulate carbon or total suspended solids can neither differentiate living from nonliving materials such as sediments, clays, or detritus; nor bacteria from the algae. Consequently, each of these variables becomes an estimator of biomass, but none can be used to define it. Biovolume does not suffer from problems of interference, but it does involve a time-consuming measurement process which requires some expertise. It must also be converted into carbon or mass units, using some conversion factor.

Two other variables commonly used as algal biomass surrogates are chlorophyll concentration and Secchi depth. The concentration of the plant pigment, chlorophyll, is often used as an indicator of trophic state. Chlorophyll is popular because it is relatively easy to measure and does not suffer from the interferences (detritus and non-algal particulates) found in the other variables. Since chlorophyll is also integral to photosynthesis, chlorophyll serves as the link between productivity (rate of carbon incorporation) and production (biomass).

Chlorophyll has drawbacks as a biomass surrogate, however. Perhaps the greatest drawback is that the amount of chlorophyll in an algal cell may vary considerably, depending on the physiological condition of the cell or the plant species. Cells that are subject to low light conditions will have more chlorophyll in them than cells exposed to high light. Different species of algae will contain differing amounts of chlorophyll (Tolstoy 1979). Despite variation, relationships between chlorophyll and algal biovolume and algal density exist, suggesting that chlorophyll changes as cell density changes (Carlson 1980; Watson, *et al.* 1992).

Secchi depth has also been widely used as a surrogate estimator of trophic state. Transparency itself is not considered to be a definer of trophic state, but transparency is influenced by algal density, and therefore can be used as an inexpensive surrogate for algal biomass. Secchi depth correlates best with algal biomass (as measured by chlorophyll) in non-colored, non-turbid situations where algae dominate the attenuation on light. However, it is subject to a number of interferences and methodological problems (See Secchi Depth chapter). Thus Secchi depth should not be used exclusively when better methods are also available.

A major oversight in the use of algal biomass as the definer of trophic state is that it ignores the presence and importance of aquatic macrophytes in the determination of the trophic state. Their omission may seriously underestimate the total plant biomass in a lake. A method to remedy this problem will be discussed later.

Hypolimnetic Oxygen

The presence or absence of oxygen in the hypolimnion of lakes is often used as a major aspect of trophic state classification. When Naumann's and Thienemann's classification schemes were combined, hypolimnetic oxygen became one of the defining characteristics of trophic state. As mentioned earlier, problems arose when morphometric factors were found to also play a major role in defining the presence or absence of hypolimnetic oxygen during the summer. Hypolimnetic temperature was also found to regulate the rate of oxygen depletion. Dissolved organic compounds also contributed to the depletion of oxygen, even in lakes of low productivity, and thus became one of the defining characteristics of dystrophy (Thienemann 1921; Naumann 1932).

Some limnologists would use the presence or absence of oxygen as the sole delimiter of the difference between oligotrophic and eutrophic lakes, regardless of the productivity or production within the lake. While it is true that oxygen depletion can have a dramatic effect on the biota and chemistry of the lake, it would be better to consider hypolimnetic anoxia as a possible result of the lake's trophic state, not the definition of it. Certainly, anoxia can result from increased productivity, but the relationship between the amount of productivity and anoxia is modified by the volume of the hypolimnion and the temperature of the hypolimnetic water. Volume and temperature have nothing to do with the trophic state concept, yet are major determinants of hypolimnetic anoxia. Thus the term, trophic state, loses much of its meaning if temperature and volume become its determinants. It seems best, therefore, to regard anoxia as a consequence of eutrophication, relying on Naumann's, rather than Thienemann's trophic concept.

Multi-Variable Definitions

In the combined Naumann-Thienemann trophic state definition, the lines between morphological factors, chemical factors, and biological structure became increasingly blurred. Naumann's original emphasis on biological production became lost in a list of "trophic state" variables, some or all of which were used to classify lakes. Some of the modern treatments of trophic state continue this "combination-of-ingredients" approach to trophic classification, declaring that trophic state is a complex aggregate of physical, chemical, and

biological variables that can only be dealt with in a multivariate manner and classified in probabilistic terms. Some of the most prevalent forms of multivariable classifications are discussed below.

Cause-Effect Combination Definitions

The simplest forms of these multifaceted definitions are those that combine a nutrient causal factor, usually phosphorus, with a biological effect factor, such as algal biomass. For example, a eutrophic lake would be a lake with high algal chlorophyll *caused by* high phosphorus concentrations or high nutrient loading. In these definitions the process of eutrophication, rather than trophic state, is being defined. For example, eutrophication might be defined as increased nutrient loading (cause) which results in increased biological productivity, plant biomass, or hypolimnetic anoxia (effect).

Carlson (1984) points out that these cause-effect definitions suffer because, in some cases, the effect is not necessarily the result of the defined cause. Zooplankton grazing or short water residence times can lower algal density despite high nutrient loads. If a lake becomes shallower with time, and macrophytes become abundant, has the lake undergone eutrophication if plant productivity has increased but external loading or internal nutrient concentrations have not?

This linking of cause and effect triggered the proliferation of Naumann's terminology, and the weaknesses and pitfalls of that approach remain. Faced with situations where the effect occurred independent of changes in the causal factor, the investigator must either ignore the contradiction, choose to classify on either the cause or the consequence, or, as did Naumann, add new names to the system. None of these options seems desirable.

Table 7.2.—General characteristics of oligotrophic and eutrophic lakes (After Rast and Lee 1987)

Variable	Oligotrophic	Eutrophic
Plant Production	Low	High
No. of Algal Species	Many	Few
Characteristic Algae	—	Blue-greens
Aquatic Rooted Plants	Sparse	Abundant
Hypolimnion Oxygen	Present	Absent
Characteristic Fish	Deep-dwelling, coldwater fish such as trout, salmon, and cisco	Surface-dwelling, warmwater fish such as pike, perch, and bass; also bottom-dwelling fish such as catfish and carp
Water Quality for Domestic and Industrial Use	Good	Poor

Classifications Based on Lake Types

One unfortunate remnant of the Naumann-Thienemann trophic state system is the belief that lakes can be divided into distinct classification groups or "lake types." Lake typology reflects the philosophy that lake types are distinct, potentially separate entities, each able to be classified according to its own characteristics (Carlson 1979). These characteristics may exhibit variation within each lake type, and may even overlap between types, but the types are considered largely distinct from one another.

Often typological classifications use a table of characteristics that would be expected for each lake type. An example of a qualitative list of characteristics is given in Table 7.2. This list provides a general impression of the trophic state of a lake or reservoir, but is difficult to use. Quantitative tables also exist, using either distinct boundaries between trophic states or overlapping ranges of values, as in Table 7.3.

Table 7.3.—A quantitative approach to lake classification (After Vollenweider and Kerekes 1980)

Variable	Oligotrophic	Mesotrophic	Eutrophic	Hypereutrophic
Total Phosphorus (µg/L)				
Mean	8.0	26.7	84.4	—
Range	3.0-17.7	10.9-95.6	16-386	750-1200
Total Nitrogen (µg/L)				
Mean	661	753	1875	—
Range	307-1630	361-1387	393-6100	—
Chlorophyll *a* (µg/L)				
Mean	1.7	4.7	14.3	—
Range	0.3-4.5	3-11	3-78	1-150
Secchi Depth (m)				
Mean	9.9	4.2	2.45	—
Range	5.4-28.3	1.5-8.1	0.8-7.0	0.4-0.5

Usually the variables on a quantitative or qualitative list are not given any priority; a lake may be classified as oligotrophic by one variable and mesotrophic or eutrophic by another. This situation often happens in deep lakes where the hypolimnion contains oxygen but there are visible algal growths in the upper waters, or in smaller lakes, where the water may be clear, but the hypolimnion is anoxic.

These indices are assumed to work no matter what the kind of lake or where it might be located. Even Naumann and Thienemann recognized that lakes vary geographically in their characteristics. As mentioned earlier, Thienemann initially thought that the classification system was only suitable for lakes in temperate climates.

These classification systems produce only a qualitative label for the reservoir, however. Labels do not lend themselves to quantification, and, therefore, to prediction. Thus, the trophic designation becomes a dead end, rather than a gateway into predicting the other aspects of the reservoir's chemical and biological condition, as was envisioned by Naumann.

Qualitative labels also suffer because the labels do not recognize variations within the type. There is a great deal of variation and change possible in any variable, be it phosphorus, chlorophyll, or biological structure, that is incorporated within the single trophic state designation of "eutrophic." Under the present trophic state classification system two lakes of the same trophic label might have chlorophyll concentrations that vary by two or fourfold. Most limnologists don't recognize this as a problem, so how could volunteers be expected to know that these lakes might function very differently?

One solution is to add more trophic categories, such as ultra-oligotrophic and hypereutrophic, but this adds more names to memorize and to characterize. Adding more categories also undermines the concept that lakes are distinct types, and tacitly acknowledges that the names are being used to arbitrarily divide a continuum.

List-of-characteristics approaches also ignore all of the other trophic types that Naumann erected. Lists such as that given in Table 7.2 could not classify a lake with a 10 cm transparency filled with silt nor a lake with highly colored water. Of course the list could be expanded to include other lake types, but the list approach was tried by Naumann and history discarded it. It does not seem necessary to recycle an approach proven to be inadequate .

Probabilistic Indices

A quantitative typological classification system was developed by a study group sponsored by the Organization for Economic Cooperation and Development (OECD). The study group apparently believed that lake types did exist, but that there was variation in the values of variables associated with any lake type. They produced a classification system based on the probability that a lake or reservoir will have a given trophic state (Fig. 7.3). For example, a lake having a total phosphorus concentration of 10 mg/m^3 in the epilimnion would have a 63% percent probability of being oligotrophic, a 26 percent chance of being mesotrophic, and a 1 percent chance of being eutrophic (Vollenweider and Kerekes 1980). Contrast this approach with that of Vollenweider (1976), where 10 mg/m^3 is used as an absolute boundary between oligotrophy and mesotrophy.

The assumed advantage of the probabilistic approach is that responses to a given variable, such as phosphorus, will vary lake-to-lake, and therefore prediction of its trophic state is best stated in probabilistic terms. This approach is analogous to the difference of predicting a single phosphorus concentration for a lake from a loading model and the presenting the probability of a predicted nutrient concentration.

However, there are several problems with the OECD classification method. First, the system is based on the premise that distinct lake types exist. The probabilistic advantage exists only if distinct trophic types actually exist. However, the criterion or criteria that designate a given trophic type are not stated. What does an oligotrophic lake look like at high phosphorus concentrations? Is it called oligotrophic because of low biomass, productivity, or, because oxygen remains in the hypolimnion?

The original designations were based on the opinions of experts, not on a single or even multiple quantitative criteria. The ranges of the curves were obtained

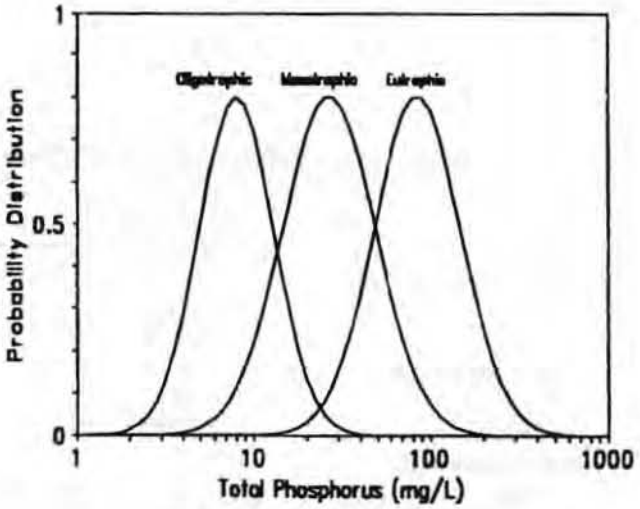


Figure 7.3—The probability distribution of trophic state categories based on average total phosphorus concentrations (Vollenweider and Kerekes 1980).

by deriving a normal curve from the mean and standard deviations of opinions of a group of experts; no lake may ever exist at the extremes of each trophic state. Setting probabilistic limits may seem more realistic than absolute limits, but only if trophic states are real, not arbitrary divisions of a continuum.

A second problem with this approach is that, as with a qualitative classification system, no effort has been made to first correlate the different variables to provide a single trophic designation. It is possible that a lake may be classified as eutrophic if phosphorus is used as the defining variable, and mesotrophic or even oligotrophic if chlorophyll is used. This dual classification is not due to variability in the values of each variable, but to the fact that the authors of the index did not examine possible empirical relationships between variables.

Finally, the end product of the index is a trophic state label. Apparently all the sources of variation are incorporated in the probability distribution; no effort is made to identify and separate out the sources. Therefore, this type of index has little predictive ability, especially outside the geographical region where it was designed. The index suffers from all the problems listed above for qualitative indices.

Quantitative Multivariable Indices

Some indices attempt to maintain the multiple variable aspect of the Naumann-Thienemann concept while adding a quantitative trophic state designation to the index. This is done by combining more than one variable into a single index value. The multivariate indices of Brezonik and Shannon (1971) and the EPA trophic state index (EPA 1974) utilize a number of variables related to trophic state, yet deviate from the classical interpretation in that they produce a continuous, numerical classification rather than typological categorizations.

The drawback of indices such as these is that several variables must be measured before a classification can be made, increasing the expense of classification. These indices also have limited utility in providing classifications if data are missing. They cannot be used for predicting future states because all of the variables cannot be predicted independently. Refer to Carlson (1979) for a detailed description and discussion of these indices.

Trophic State: An Evaluation

Numerous definitions of trophic state now exist in the literature. There are so many, in fact, that the entire concept is at risk of becoming a "non-concept." Carlson (1984) comments that this situation would be a real loss to limnology, because the concept, if not the classification, still has the potential to communicate valuable information to scientists and to the lay public. Perhaps even more importantly, it has the ability to aid in the organization of thoughts, ideas, and research about lakes. There are good reasons to think that trophic state remains a critical organizing concept in the study of lakes.

Imagine asking questions about fish production without referring to the base of food chain, or discussing restoration without reference to the watershed. Most investigators will preface their studies with a description of the trophic state of a lake, assuming that all the readers will understand the meaning of the term. Trophic classification is a necessary statement that allows us to locate the lake in the production continuum. From that location we can make predictions of structure and function of that system. Without that location and the ensuing ability to estimate further attributes of the system, each lake becomes an independent entity, devoid of all connection to previous information and knowledge of other lakes, or even previous studies. This certainly is not the way most limnologists think of lakes and limnology.

Any attempt to resurrect and modernize the Naumann-Thienemann trophic concept will ultimately be met with ever-increasing frustration similar to that found by past investigators. Some classifiers have chosen to ignore the possible variations in biological structure caused by factors other than nutrients, producing a single-axis trophic scale based on nutrients alone. Unfortunately, the biological structure of all lakes does not respond in a linear manner to nutrient additions. If the investigator chooses to include other abiotic axes as well, he risks the proliferation of lake types, overlapping and contradictory classifications, and a system as cumbersome and as susceptible to collapse as was the Naumann-Thienemann model.

At the risk of producing just such a trophic classification system, below are several statements which may be controversial, but might lead to a usable and useful classification system.

1. There are no such thing as lake types.

Lake typology is a historical artifact that has no reality in lakes. No study supports the concept that lakes or their biota have a distinct identity or wholeness that separates one lake type from another. Most abiotic variables change continuously along gradients, and production and the biological structure change continuously as well. The propensity to lump lakes into groups is a quirk of the human mind, not a necessity determined by Nature.

The presence or absence of hypolimnetic oxygen comes closest to a single separating variable because along the entire axis of possible nutrient and algal concentrations, anoxia develops relatively rapidly

(within one doubling of phosphorus concentration). However, even the development of hypolimnetic anoxia is a continuous, rather than a discrete process, appearing to be abrupt only when compared to the entire spectrum of possible nutrient or biomass concentrations. In any given lake, the process is defined as the rate at which oxygen is depleted, not the mere presence or absence of oxygen.

Dividing a continuum into subgroups is done for convenience. However, once classes have been established, they take on a life and reality of their own, as happened with Naumann's classification. It might be best to abandon the trophic terminology all together, relying on quantitative classifications instead. It is more difficult to ascribe groupings to a numerical scale.

2. Lake typology and trophic classification are not the same thing.

As the original trophic classification developed, the original classification of production became less and less obvious as the principal variable of interest. For the most part, the classification exercise became one of classifying lakes rather than classifying the amount of biological production. All the factors that affect production became incorporated into the classification; nutrients, light, morphology, turbidity, location, etc.

Lakes can be described in many ways including depth, thermal structure, water chemistry, size, etc. Trophic state, however defined, is only one possible manner of classifying lakes. Naumann's terminology should have been specific to production or the factors that limit production. An acid lake is only acidotrophic if acid limits production. A turbid lake is argillotrophic only if the turbidity limits plant biomass. Unfortunately, Naumann's classification seemed to confuse, then fuse, trophic description with the description of the lake. If trophic classification is looked upon as reflecting one particular (and important) aspect of a lake rather than as some sort of total classification, the trophic concept can be kept simple and in perspective.

3. Production is the simplest and most useful definition of trophic state.

Much of what is written in this chapter alludes to the fact that no single variable or even concept has exclusive historical claim to be the "true" definition of trophic state. Even in Naumann's time, and apparently with his blessing, the concept changed and became more elaborate. The fact that the classification finally collapsed indicates that the scheme had become too elaborate and inclusive. It no longer reflected a chief purpose of classification—utility. Many biological classifications, including binomial nomenclature, are, at least in part, artificial. Being largely a construct of the human mind, they are simple tools in organizing and communicating ideas. Trophic state definitions are no different; they must organize knowledge and they must communicate that knowledge.

It has already been explained why nutrients should not be used as the basis for a trophic state definition. It would not be historically accurate, but more importantly, neither phosphorus nor nitrogen is the primary object of interest in lake classification or management. They only gain their importance as they relate to or affect the biological situation in the lake. The fact that a lake has high concentrations of phosphorus is only of interest if that phosphorus translates (or potentially translates) into high amounts of algae or macrophytes.

Definitions based on either biomass or productivity can draw upon a long tradition of classifying aquatic as well as terrestrial ecosystems by amount of organic matter in the system and / or the rate of entry of energy into the system. The reasons why productivity would not be the best trophic state variable have already been explained; it is difficult to measure and to interpret.

Biomass-related trophic state definitions are consistent with systems terminology's use of the term "state," as a measure of the amount in a system at a given point in time. Although the term "biomass" itself must be operationally defined, it can be estimated by a number of techniques, from Secchi depth to adenosine triphosphate (ATP) analysis. With such a variety of available techniques, a technique appropriate to each budget or situation can usually be found. Biomass also lends itself to multi-lake surveys, where there is often insufficient time to do intensive productivity analyses. Finally, biomass is an approximate measure of the problems that plague lakes. Few citizens complain about the productivity of their lake and fewer yet lodge complaints about phosphorus. A biomass-related trophic state definition places the emphasis of the classification on the problem (e.g., too much algae or too many macrophytes which, in turn, interfere with lake uses) rather than on any potential cause.

4. Eutrophication is nothing more (or less) than the movement of a lake's production along a continuum in a direction from oligotrophy towards eutrophy.

In Naumann's trophic state classification, the terms oligotrophic and eutrophic marked two classes of lakes along the nitrogen and phosphorus axis. As a lake became eutrophic, the process was called eutrophication. Conversely, if a eutrophic lake became oligotrophic, the process could be called *oligotrophication*. Eutrophication is not a mysterious process or even one necessarily linked to nutrient change. It is simply a directional movement.

Because production might be increased or decreased by factors other than nutrients, it is more appropriate

to use production as the central axis rather than nutrients. Because production is most often related to changes in nutrient status in the lake, eutrophication and oligotrophication are processes often caused by changes in the lake's nutrient content, and, ultimately, nutrient loading to the lake. However, the change could just as well take place if another limiting factor, such as turbidity, were removed, as long as sufficient nutrients were available to allow increased plant production. Eutrophication would have occurred even if there had been no change in loading, or even nutrient status.

5. **Biological structure of lakes is affected by many factors other than nutrients and should not be used to define trophic state.**

Unlike production, the biological structure of a lake is much more susceptible to change along gradients other than nutrients. As mentioned above, one mechanism to maximizing production and productivity is a change in the dominant species to those best suited to the ambient conditions. Changes in species can be expected as a lake becomes acidic or colored with humic substances. In other cases, intolerance to prevailing conditions will eliminate whole groups of organisms, as was found in acidic lakes, where vertebrates were largely missing. In these instances, whole trophic pathways will be altered or eliminated. The same alteration of food webs may also be found in other environments such as turbid reservoirs and lakes.

If trophic state were defined on the basis of biological structure rather than on production, it might be expected to change as a function of the intensity of a number of environmental factors. Change pH, nutrient loading, hypolimnetic anoxia, or salinity, and the biological structure (and therefore trophic state) will be expected to change as well.

Clearly, structural changes will not fit neatly into lake types because changes will probably occur gradually along any one or more environmental axis. Any sort of typological classification would be impossible; the name game would be overwhelming. Probably some sort of multivariate quantitative classification would be most useful. Whatever the approach that is used, the ability to classify the biological structure of lakes is clearly needed.

Biological structure has a legitimate and compelling claim to the concept of trophic state. Although not the original trophic state definition, biological structure gained importance in later classifications, especially after the merger of the Naumann and Thienemann classifications. Naumann considered that many of the lake types, such as acidotrophy or argillotrophy, had equal productivity but distinctly different biological communities.

In a subtle way, Naumann made a distinction between production and structure. He split the classification into two typologies, one describing the water (*Wasser*) and the other the water body (*Gewasser*). In the first he placed production, in the second, biological structure. What he did was to distinguish between variables such as production and productivity and those relating to biological species (structure). This distinction is useful because they are really two distinct but related aspects of biological systems.

Production and productivity relate to the amount and rate at which energy enters the lake system. They share with other variables, such as nutrient concentration, the attribute that they are conservative, that one can always account for the income, outgo, and location of every kilocalorie or gram. People can write energy or nutrient budgets for them and predict how much energy or nutrient will be used in the future. They are, however, fundamentally different from biological structure. Biological structure is based on biological species. A species becomes present in the community when a single organism arrives, but the loss of a single individual does not mean the species is absent. For a species to be absent again, it must go extinct. A species cannot be weighed; it may consist of a single individual weighing a kilogram, or the total weight of millions of individuals of a species may weigh only a thousandth of a gram.

Species are a member of a category called *information*. Information is *non-conservative*; that is, you can't weigh or account for information in the manner you can with materials and energy. Two cents worth of information can be given to one person or a million people, each receives the same amount of information. A single individual of a species brings far more than two cents worth of information into an ecosystem. That information is present whether there be one or a million individuals of that species. This non-conservative nature of information makes it almost impossible to predict species structure from energy or material variables. A kilogram of organic material may contain a single or a thousand different species, arranged in an almost infinite number of trophic web arrangements.

More important yet, biological structure has the unique ability to adapt, evolve, and change to fit the surrounding environment. Given time, it is theoretically possible for life to evolve for the maximum utilization of nutrients and energy even in the most extreme environment. The fact that there are latitudinal differences in productivity attests to the fact that evolution has limits. Within broad thermodynamic constraints, however, evolution can produce a dazzling kaleidoscope of possible biological communities.

If biological structure can be so diverse, it could be successfully argued that each lake ecosystem is to

some extent comprised of a unique community structure. If each lake is different from the next, then any classification ultimately will be frustrated by this range of possibilities. Alternatively, classification may attempt to force the diversity of structure into an artificial classification scheme, overlooking the small differences, preferring to see the similarities.

Biological structure classification is distinctly different from what Naumann originally intended (i.e., the linking of watershed characteristics to the biological production of the water). The original trophic scheme was deeply involved in the factors of production, not structure. It is to Naumann's credit that he insisted that production would, in turn, affect structure, and ultimately the ontogeny of the lake itself. Classification based on biological structure would have to include numerous measures that affect biological structure, but which are unimportant to Naumann's concept of factors that affect production.

In the final analysis, it is plant production (biomass) and not biological structure that should be used as the definition of trophic state. It has historical consistency, is relatively easy to measure, and is a measurement that conveys meaning to limnologists, lake managers, and the public. Biological structure should be considered as a separate classification. Structure is important and far more vulnerable to environmental stresses than production. The ability to classify and track structure is an important aspect of limnology because structural changes may ultimately affect function. It deserves more attention, but it should not be called trophic state. Call it biodiversity, biological structure, biotype, or whatever, but just not trophic state.

Trophic State—Volunteer Programs

The coordinator of a volunteer program is in a unique position to understand the need for a clearly-defined, useful, and communicable definition for trophic state. The coordinator serves at least three basic constituencies. First, monitoring programs are often mandated by the state governments. In some states coordinators are required to obtain certain information to satisfy 305b reporting requirements and to qualify for 314 Clean Lakes funds. The trophic state determination is needed for both programs. The more complex the definition of trophic state, the more information that will be required to classify lakes. If the definition is too complex, data requirements will be beyond the scope and budget of most volunteer monitoring programs.

Second, volunteers expect information, not labels. They will be poorly served for their time and effort if all they receive is a comment that their lake is eutrophic. What does this term mean to the volunteer? Is the lake in good or bad condition? Should it be restored or protected? What is the prognosis for recreation and other uses? It would be better if the definition reflected a single aspect of the lake very well rather than present a combined variety of factors that does not convey quality information.

Third, the coordinator, or those that use the volunteer data, must make management decisions. Decisions require information. However, if the goal of the program is serving the data collection needs of a classification system, that classification had better provide more information than just a name. Certainly trophic state is only one facet of what should be called water quality, but it would be ideal if the definition described an important aspect of a management program.

The trophic state model presented above is amenable to a tiered level-of-effort approach which is compatible with the funding and goals of many volunteer programs. Most programs begin with a Secchi depth monitoring program because it is easy to train volunteers in the disk's operation and because Secchi disks are relatively inexpensive. Since Secchi depth is also an indicator of algal biomass, it is also possible to make estimates of trophic state, albeit with considerable uncertainty.

As a program matures, chlorophyll pigments and total phosphorus are added to provide a better estimate of biomass. Later in this chapter, a method will be given that uses Secchi depth, chlorophyll, and total phosphorus to make inferences about potential limitation. Addition of total nitrogen and estimates of macrophyte biomass would complete this suite of variables that would completely estimate trophic state, as defined by plant biomass, and the nutrient factors that often cause that trophic state.

Additional sampling by volunteers could provide information on biological structure. Samples of macrophytes, phytoplankton, and zooplankton could be obtained by the volunteer and shipped to a laboratory for identification. Creel censuses or careful records of fishing success by the volunteers might give some indication of fish community structure.

From these data, a picture of a lake's trophic and biological structure will emerge. From that picture, it may be possible to ask questions of causation, and a watershed monitoring program could be initiated. Volunteers could do much of the work in a watershed monitoring program and the information gathered could be very useful in managing the lake.

Finally, the information can be combined into a regional picture of trophic state. This regional aspect of trophic state was initially envisioned by Naumann and today can serve as a goal of statewide monitoring. Its use as a method for answering and generating questions has yet to be fully explored.

A Production-Based Trophic State Index (TSI)

One frequently used biomass-related trophic state indices is that of Carlson (1977). It is relatively simple to use, requires a minimum of data, and is generally easy to understand, both in theory and use. It is numerical, but the traditional nutrient-related trophic state categories fit into the scheme. It seems to be ideal for use in volunteer programs.

In accordance with the definition of trophic state given above, this index (TSI) uses algal biomass as the basis for trophic state classification. Three variables — chlorophyll pigments, Secchi depth, and total phosphorus — independently estimate algal biomass. Unlike Naumann's typological classification of trophic state, the index reflects a continuum of "states." There are no lake "types." The trophic continuum is divided into units based on a base-2 logarithmic transformation of Secchi depth, each 10-unit division of the index representing a halving or doubling of Secchi depth. Because total phosphorus often correlates with transparency, a doubling of the total phosphorus often corresponds to a halving of Secchi depth. Chlorophyll pigments double every 7 units rather than every 10 units (Carlson 1980).

The range of the index is from approximately zero to 100, although the index theoretically has no lower or upper bounds. The index has the advantage over the use of raw variables in that it is easier to memorize units of 10 rather than the decimal fractions of raw phosphorus or chlorophyll values. An early version of the index was based on a scale of one to ten, but it became tempting to add 1, 2, or more numbers after the decimal. For this reason, the scale was multiplied by ten to discourage any illusory precision obtained by using more than whole numbers.

The logarithmic transformation of the data normalizes the skewed data distribution, allowing the use of parametric statistics (mean, standard deviation, parametric comparison tests). This facilitates not only comparison and data reduction, but communication as well, because the user does not need to resort to graphs with logarithmic axes.

The three index variables are interrelated by linear regression models, and should produce the same index value for a given combination of variable values. Any of the three variables can therefore theoretically be used to classify a lake or reservoir. This is particularly useful in citizen lake monitoring programs, where Secchi depth is often the only variable that can be inexpensively measured. For the purpose of classification, priority is given to chlorophyll, because this variable is the most accurate of the three at predicting algal biomass. According to Carlson (1977), total phosphorus may be better than chlorophyll at predicting summer trophic state from winter samples, and transparency should only be used if there are no better methods available.

Using the TSI

The index is relatively simple to calculate and to use. Three equations are used: Secchi disk, TSI(SD); chlorophyll pigments, TSI(CHL); and total phosphorus, TSI(TP). The original Secchi depth equation in Carlson (1977), reproduced below looks forbidding, but illustrates how the index was constructed.

$$TSI\,(SD) = 10\left[6 - \frac{\ln SD}{\ln 2}\right] \qquad (7.1)$$

The basic Secchi disk index was constructed from doublings and halvings of Secchi disk transparency. The base index value is a Secchi depth of 1 meter, the logarithm of which is zero.

$$\ln 1 = 0$$
$$6 - 0 = 6$$
$$10 * 6 = 60$$

Therefore, the TSI of a 1 meter Secchi depth is 60. If the Secchi depth were 2 meters,

$$\ln 2 \,/\, \ln 2 = 1$$
$$6 - 1 = 5$$
$$10 * 5 = 50$$

The indices for the chlorophyll and total phosphorus are derived in a similar manner, but, instead of a Secchi depth value in the numerator, the empirical relationship between chlorophyll or total phosphorus and Secchi depth is given instead.

$$TSI\,(CHL) = 10\left[6 - \frac{2.04 - 0.68 \ln CHL}{\ln 2}\right] \qquad (7.2)$$

$$TSI\,(TP) = 10\left[6 - \frac{\ln (48/TP)}{\ln 2}\right] \qquad (7.3)$$

The above forms of the equations may illustrate how the indices were derived, but they can be simplified for everyday use. The simplified equations are below:

$$TSI(SD) = 60 - 14.41 \ln(SD) \qquad (7.4)$$

$$TSI(CHL) = 9.81 \ln(CHL) + 30.6 \qquad (7.5)$$

$$TSI(TP) = 14.42 \ln(TP) + 4.15 \qquad (7.6)$$

Volunteers do not need the equations to calculate their own trophic state index if supplied with a graph such as illustrated in Fig. 7.4. Using their Secchi depth values, volunteers can estimate the trophic state index and plot the seasonal fluctuations in trophic state. It could be suggested that they compare the index values against a chart of predicted trophic characteristics (Table 7.4). In this manner they can see that the trophic state of a lake affects numerous characteristics of the lake, not just transparency.

Averaging Values

A misuse of the TSI values is to average the three TSI values, producing a single TSI value (Osgood 1983; Kratzer and Brezonik 1981). Perhaps this is just a natural tendency for humans to seek the central tendency, or it might reflect the residual effects of the multivariable trophic state concept. Whatever the reason, averaging makes no sense at all. The index is predicated on the idea that it is predicting algal biomass. Chlorophyll is a better predictor than either of the other two indices. Although transparency and phosphorus may co-vary with trophic state, the changes in transparency are caused by changes in algal biomass and total phosphorus may or may not be strongly related to algal biomass. Neither transparency nor phosphorus are independent estimators of trophic state. Using transparency or phosphorus as an estimator of chlorophyll is very different than assuming equal and independent status of the variables.

Apparently the availability of more than one value invites the averaging of the values (Osgood 1980). Carlson (1980) emphasized that the averaging of chlorophyll with the predicted chlorophyll based on Secchi depth is equivalent to assuming that temperature is better estimated by averaging the reading from a thermometer with the number of cricket chirps per minute. Secchi depth should be used as a surrogate, not covariate, of chlorophyll.

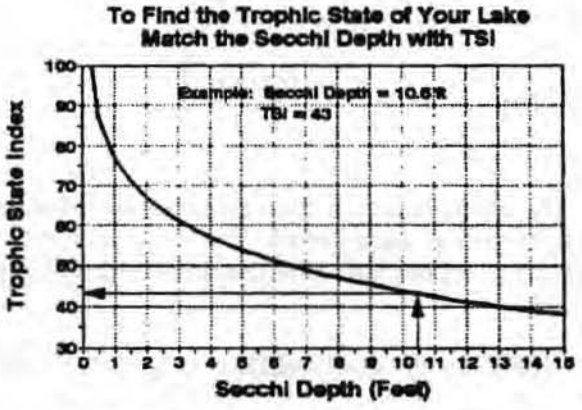


Figure 7.4—A graph that can be used by volunteers to calculate trophic state using Secchi depth values.

Beyond Classification

A major strength of this index is that the interrelationships between variables can be used to identify certain conditions in the lake or reservoir that are related to the factors that limit algal biomass or affect the measured variables. When more than one of the three variables are measured, it is possible that different index values will be obtained. Because the relationships between the variables was originally derived from regression relationships and the correlations were not perfect, some variability between the index values is to be expected. However, in some situations the variation is not random and factors interfering with the empirical relationship can be identified. These deviations of the total phosphorus or the Secchi depth index from the chlorophyll index can be used to identify errors in collection or analysis or real deviations from the "standard" expected values (Carlson 1980b). Some possible interpretations of deviations of the index values are given in Table 7.5 (Carlson 1983).

The simplest way to use the index for comparison of variables is to plot the seasonal trends of each of the individual indices. If every TSI value for each variable is similar and tracks each other, then you know that the lake is probably phosphorus limited (TN/TP > 33; Carlson 1992) and that most of the attenuation of light is by algae.

In some lakes, the indices do not correspond throughout the season. In these cases, something very basic must be affecting the relationships between the variables. The problem may be as simple as the data were calculated incorrectly or that a measurement was done in a manner that produced different values. For example, if an extractant other than acetone is used for chlorophyll analysis, a greater amount of chlorophyll might be extracted from each cell, affecting the chlorophyll relationship with the other variables. If a volunteer incorrectly measures Secchi depth, a systematic deviation might also occur.

After methodological errors can be ruled out, remaining systematic seasonal deviations may be caused by interfering factors or non-measured limiting factors. Chlorophyll and Secchi depth indices might rise above the phosphorus index, suggesting that the algae are becoming increasingly phosphorus limited. In other lakes or during the season, the chlorophyll and transparency indices may be close together, but both will fall below the phosphorus line (Fig. 7.5A). This might suggest that the algae are nitrogen-limited or at least limited by some other factor than phosphorus. Intense zooplankton grazing, for example, may cause the chlorophyll and Secchi depth indices to fall below the phosphorus index as the zooplankton remove algal cells from the water (Fig. 7.6A).

In turbid lakes, it is common to see a close relationship between the total phosphorus TSI and the Secchi

Table 7.4—Predicted trophic characteristics relating to TSI values

TSI Value	Attributes	Water Supply	Recreation	Fisheries
TSI's <30	Classical oligotrophy: clear water, oxygenated hypolimnion.			Salmonoid fisheries in deeper lakes.
TSI 30-40	Hypolimnion in shallower lakes may become anoxic in summer.			
TSI 40-50	Water moderately clear, but increasing probability of hypolimnetic anoxia in summer.	Iron and manganese problems. Raw water has noticeable odor, THM precursors exceed 0.1 mg/L, and turbidity exceeds 1 NTU.		Loss of salmonoid species because of hypolimnetic anoxia.
TSI 50-60	Classical eutrophy: Decreased transparency, anoxic hypolimnia during the summer, macrophyte problems may be evident.	Iron and manganese, taste and odor, turbidity and THM problems worsen.		Warm-water fisheries only. Bass and perch may be dominant.
TSI 60-70	Blue-green algae dominate during the summer, algal scums probable, considerable macrophyte problems.		Boating difficult because of weeds, transparency and algal scums discourage swimming.	
TSI 70-80	Dense algae and macrophytes in summer.			Winter fish kills possible in shallower lakes.
TSI >80	Algal scums, few macrophtyes.			Rough fish dominate, summer fish kills possible.

Table 7.5—Interpretations of deviations from typical conditions associated with TSI values

TSI Relationships	Possible Interpretation
TSI(CHL) = TSI(SD)	Algae dominate light attenuation
TSI(CHL) > TSI(SD)	Large particulates, such as Aphanizomenon flakes, dominate
TSI(TP) = TSI(SD) > TSI(CHL)	Non-algal particulate or dissolved color dominate light attenuation
TSI(SD) = TSI(CHL) >= TSI(TP)	Phosphorus limits algal biomass (TN/TP ratio greater than 33:1)
TSI(TP) > TSI(CHL)=TSI(SD)	Zooplankton grazing, nitrogen, or some factor other than phosphorus limits algal biomass

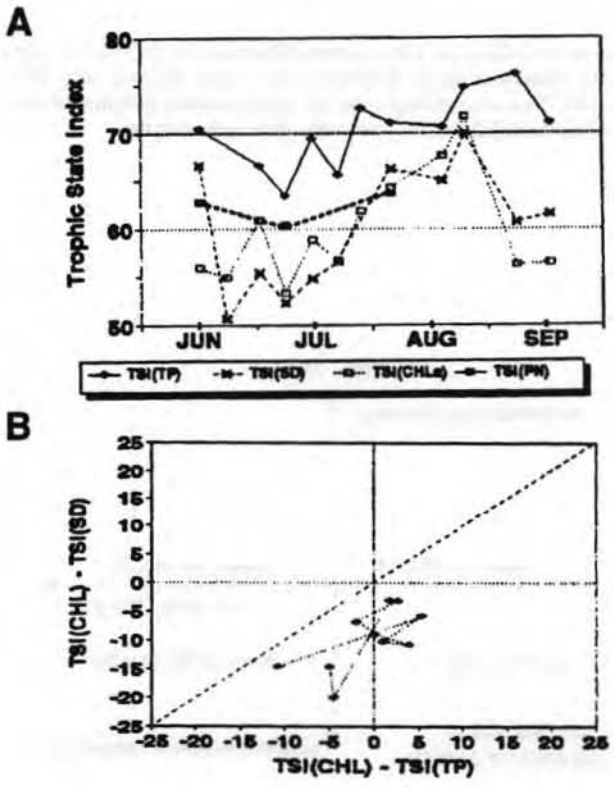


Figure 7.5—*Graph A* The seasonal fluctuations in Trophic State Index relationships in Lake LaGrange, NY. The TSI(PN) index is derived using the phosphorus-nitrogen equations of Walker (1985). *Graph B* The same data plotted as differences of the phosphorus and Secchi depth indices from the chlorophyll index.

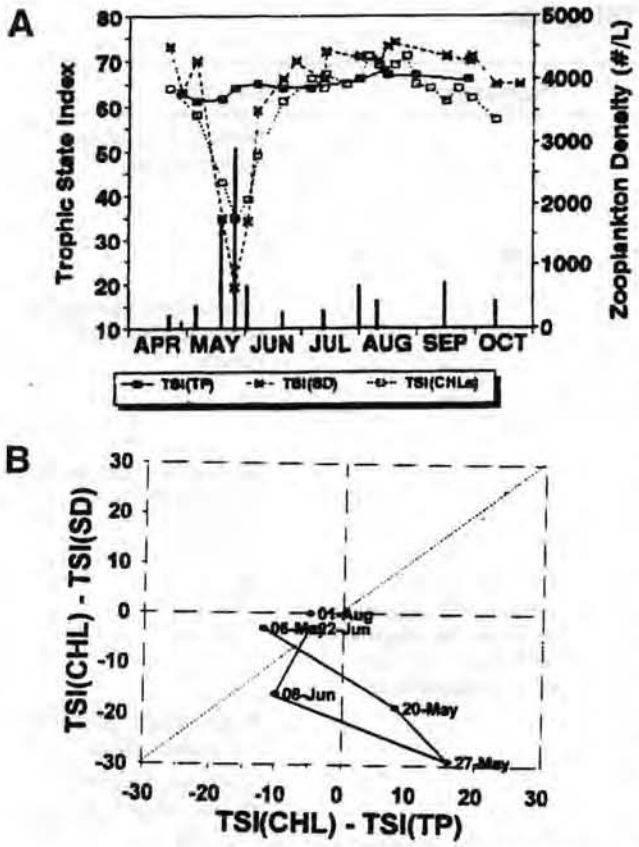


Figure 7.6—*Graph A* The seasonal fluctuations in Trophic State Index relationships in Halsted's Bay, Lake Minnetonka, MN. *Graph B* The same data plotted as differences of the phosphorus and Secchi depth indices from the chlorophyll index.

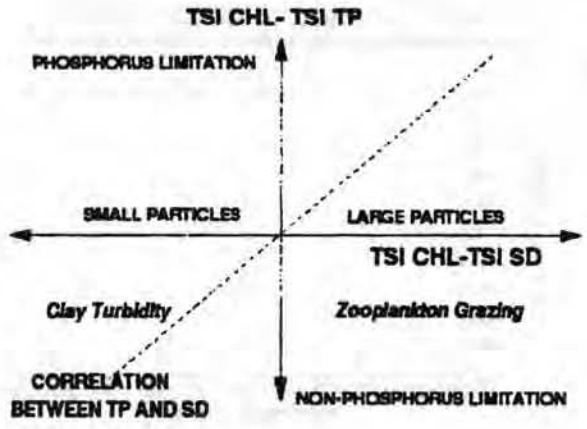


Figure 7.7—An illustration of possible causes for deviations on a multiple plot of deviations of TSI(TP) and TSI(SD) from TSI(CHL).

depth TSI, while the chlorophyll index falls 10 or 20 units below the others. Clay particles contain phosphorus, and therefore lakes with heavy clay turbidity will have the phosphorus correlated with the clay turbidity, while the algae are neither able to utilize all the phosphorus nor contribute significantly to the light attenuation. This relationship of the variables does not necessarily mean that the algae is limited by light, only that not all the measured phosphorus is being utilized by the algae.

A different way of looking at deviations is reported in Carlson (1992). If both of the deviations, TSI(CHL) - TSI(TP) and TSI(CHL) - TSI(SD), are simultaneously plotted on a single graph, it is possible to identify some of these systematic deviations. The possibilities are illustrated in Fig. 7.7.

If TSI (CHL) - TSI (TP) is plotted on the vertical axis, then points below the X-axis would be associated situations where chlorophyll is under-predicted by total phosphorus, i.e. situations where phosphorus may not be limiting chlorophyll (Fig. 7.5 B). Carlson (1992) reported that this zero line is related to total nitrogen to total phosphorus (TN/TP) ratios greater than 33:1. Phosphorus is usually thought to become limiting at a TN/TP ratio of 10:1, therefore slight deviations below the zero line would not indicate nitrogen limitation. A better interpretation would be that the greater the negative deviation, the greater the probability of something other than phosphorus limits algal growth. A combined phosphorus and nitrogen TSI deviation could also be used for this axis to eliminate the effects of nitrogen as well as phosphorus limitation. As points go above the zero axis, it would suggest increasing possibility of phosphorus limitation.

Points lying to the right of the Y-axis indicate situations where the transparency is greater than expected from the chlorophyll index. These deviations may occur if large particulates, such as blue-green algae, dominate, and transparency is less affected by the particulates. Deviations to the right may also occur if zooplankton grazing removes smaller particles and leaves only large forms (Fig 7.6 B). Points to the left of the Y-axis would be related to situations where transparency is dominated by non-algal factors such as color or turbidity or where very small particles predominate.

Points lying near the diagonal to the left of the origin indicate situations where phosphorus and transparency are correlated, but chlorophyll is not. Points on or near this line would be found in turbid situations where phosphorus is bound to clay particles and therefore turbidity and phosphorus are related, but chlorophyll is not. This is illustrated using data collected from Lake Rockwell, Ohio (Cooke and Carlson 1987). The data were collected at a number of stations rang-

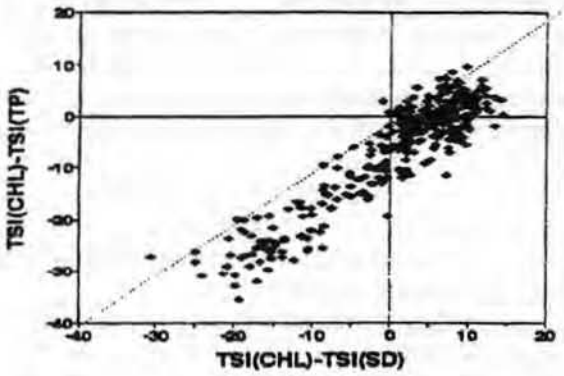


Figure 7.8—The TSI deviation plot at several stations in Lake Rockwell, OH.

ing from the incoming river to the dam. The plot (Fig. 7.8) illustrates the effect of non-algal turbidity: the most deviant points are the river stations. As the water passes down the reservoir the non-algal turbidity is apparently lost, and the points fall closer to the 0,0 intercept. That the data do not pass directly through the 0,0 intercept and instead intercept the zero nutrient axis at a negative transparency deviation would suggest that the particles in the water are large and therefore produce a "sieve" effect (Edmonson 1980; Havens and Heath 1991).

This form of graph collapses the deviations of the Secchi depth TSI onto the graph of the other deviations, allowing simultaneous viewing of the deviations of all three indices. The spatial location of the data for a single lake or for a number of lakes can therefore be used to infer possible relationships between the three variables. This use of the index is still being developed but holds considerable promise in the interpretation of data.

Adding Indices

Nitrogen

Other indices have been constructed to be used with the basic three. Since nitrogen limitation still classifies a lake along Naumann's nutrient axis, the effect of nitrogen limitation can be estimated by having a companion index to the Total Phosphorus TSI. Such an index was constructed by Kratzer and Brezonik (1981) using data from the National Eutrophication Survey on Florida lakes. This index is calculated using the formula:

$$TSI(TN) = 54.45 + 14.43 \ln(TN) \quad (7.7)$$

(Nitrogen values must be in units of mg/L.)

Carlson (1992) incorporated both nitrogen and phosphorus into the same nutrient equation, using the model of nitrogen-phosphorus empirical model of Walker (1985). Walker's model estimates chlorophyll concentrations based on a combination variable, X_{PN}, which is a combination of total nitrogen (µg/L) and total phosphorus (µg/L). Carlson (1992) then transformed the chlorophyll into a TSI index equation.

$$X_{pn} = \left[p^{-2} + \left[\frac{N-150}{12} \right]^{-2} \right]^{-0.5} \quad (7.8)$$

$$\log CHL = -0.7 + 1.25 \log(X_{pn}) \quad (7.9)$$

$$TSI(PN) = 9.81 \ln(10^{CHL_{pn}}) + 30.6 \quad (7.10)$$

The indices of Kratzer and Brezonik were designed to be used in nitrogen-limiting conditions while Walker's model was designed to be used under both nitrogen and phosphorus limiting conditions. In reality, both models are relatively insensitive to the nitrogen:phosphorus ratio (Fig. 7.9 A & B), while the phosphorus TSI of Carlson deviates at low nitrogen phosphorus ratios (Fig. 7.9 C). This suggests that a nitrogen index value might be a more universally applicable nutrient index than a phosphorus index, but it also means that a correspondence of the nitrogen index with the chlorophyll index cannot be used to indicate nitrogen limitation. If, however, nitrogen and phosphorus indices were plotted at the same time, then a deviation of only the phosphorus index might indicate nitrogen limitation, while deviations of both nitrogen and phosphorus indices might indicate situations where nitrogen or phosphorus are not limiting.

Macrophytes

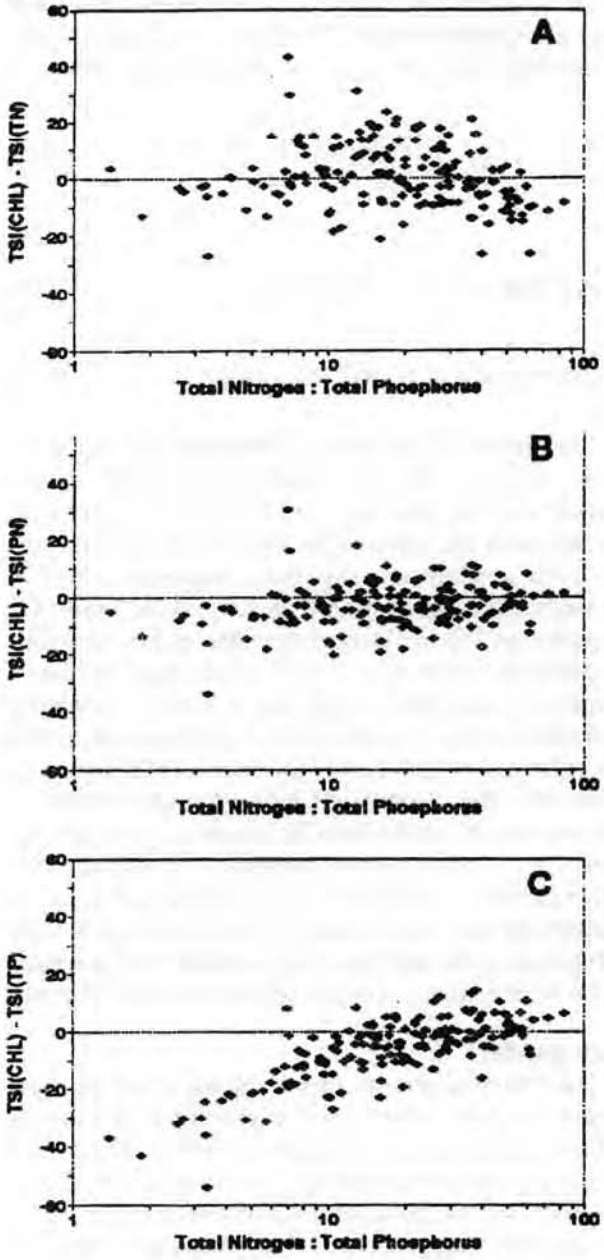
The TSI in its present form is based solely on algal biomass. It is therefore blind to macrophyte biomass and may, therefore, underestimate the trophic state of macrophyte-dominated lakes. This is a serious drawback that needs to be addressed. The solution could be very simple. Canfield *et al.* (1983) proposed a method to measure the total phosphorus content of lakes. The total macrophyte biomass in the lake is estimated by

$$TSMB = SA \times C \times B \quad (7.11)$$

where TSMB = total submersed macrophyte biomass, SA = lake surface area, C = percent cover of submersed aquatic macrophytes, and B = average biomass collected with a sampler.

Canfield *et al.* (1983) estimated the total phosphorus in plant biomass based on the phosphorus in each species and the relative abundance of each species. The total phosphorus content of the lake was obtained by adding the amount of phosphorus in the macrophytes

Figure 7.9—Deviations of chlorophyll indices as predicted by: *Graph A*, nitrogen (Kratzer and Brezonic 1981); *Graph B*, phosphorus and nitrogen (Walker 1985); and *Graph C*, phosphorus (Carlson 1977).



to the amount estimated to be in the water column. There seems to be no reason why the same approach could not be used to measure total plant biomass or chlorophyll. If it were used, trophic state could include both macrophytes and algae, and have internally consistent units.

Trophic State and Water Quality

An unfortunate misconception concerning trophic state is that the term is synonymous with the concept of water quality. Although the concepts are related, they should not be used interchangeably. Trophic state is an absolute scale that describes the biological condition of a lake or reservoir. Whether trophic state is defined by biomass, nutrients, or biological structure, the scale is a division of that variable, and is not subject to change because of the attitude of the observer. An oligotrophic or a eutrophic lake has attributes of production that remain constant no matter what the use of the water or where the lake is located. For the trophic state terms to have meaning at all, they must be applicable in any situation in any location.

Water quality, on the other hand, is a term used to describe the condition of a water body in relation to human needs or values. Quality is not an absolute; the terms "good" or "poor" water quality only have meaning relative to the use of the water and the attitude of the user. An oligotrophic lake might have good water quality for swimming but be considered poor water quality for bass fishing. Confusion can ensue when the term trophic state is used to infer quality.

Suppose, for example, that a manager were to establish fishing goals based on trophic state. Generally fish yield increases as the production of the lake increases (Fig. 7.10). However, there may be changes in the dominant fish species as a lake eutrophies (Oglesby, *et al.* 1987). In northern lakes, salmonids might dominate in clear lakes having oxygenated hypolimnia. When production increases to the point where the hypolimnion becomes anoxic, then salmonids may disappear, to be replaced by percids, then centrarchids, and finally rough fish such as carp or bullheads. If a fisheries manager wished to manage all lakes based on fish production, then the greener the lake the better. However, what is meant by good water quality would be different for a person wanting to catch lake trout than a person wanting only bass. In fisheries management, the relationship between fish production and fish community structure and trophic state do not

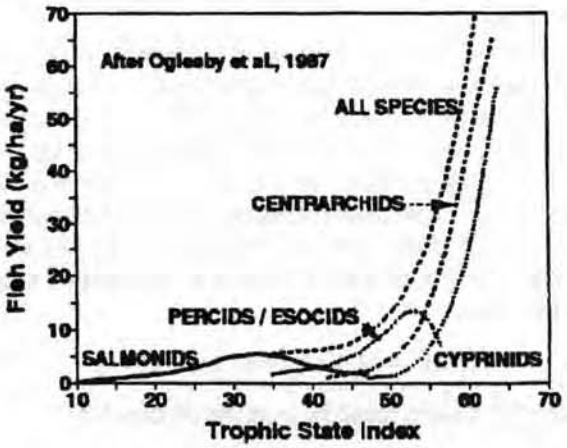
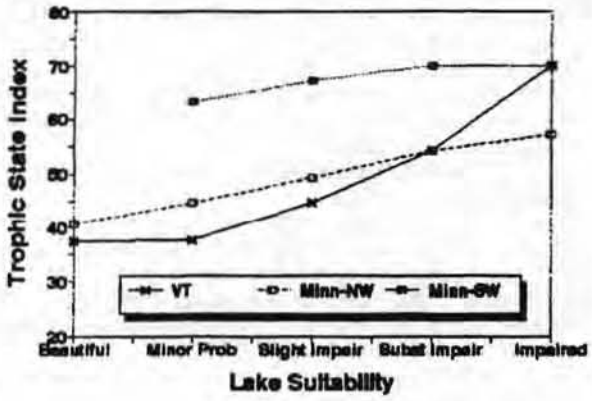


Figure 7.10—Changes in yield of several fish groups as related to trophic state (after Oglesby, *et. al.* 1987).

Figure 7.11—The influence of geographical location to volunteer perception of the water quality of their lake (after Smeltzer and Heiskary 1990).



change. What changes is the perception of what is good or bad water quality. In this case, the meaning of quality water heavily depends on the goals and expectations of the fishery and the fishermen.

Multiple use situations can cause numerous conflicts because of differing perceptions of water quality by different users. Fishermen may want the optimal water quality for their particular species of game fish, boaters will want to minimize weeds, swimmers will want to see their feet. Other users, such as drinking water utilities, may want the clearest water possible, but ignore weeds completely. Vant and Davies-Colley (1988), for example, found that lakes in New Zealand ceased to be acceptable for swimming at Secchi depths less than one meter, but Secchi depth apparently did not affect fishing, passive recreation (relaxation/observation/picnics/camping), sailing, or power boating. For each use, the trophic spectrum is being referred to, but the needs of the users, and thus the perception of quality at any given trophic state, vary considerably.

Attitude about water quality is also affected by the general background of the user. General background means the attitude of the user that is related to his or her upbringing, geographical location, and virtually all attitudes that the user brings to lake evaluation other than that of a user. In a study of attitudes about water quality, Smeltzer and Heiskary (1990) queried volunteers as to whether their lakes were beautiful or if enjoyment was slightly impaired, substantially reduced, or nearly impossible. They found that the volunteer responses varied geographically (Fig. 7.11). In Vermont and in the northeastern part of Minnesota, volunteers were more sensitive to changes in trophic state. In the agricultural region of southwest Minnesota, lakes that were considered to have minor problems would have been considered impaired in the other regions. The lesson here is that regional attitudes tend to judge "good" versus "poor" water quality.

Recommendations

Trophic state determination is an important aspect of lake surveys. Trophic state is not the same thing as water quality, but trophic state certainly is one aspect of water quality. Of particular interest for volunteer programs is that trophic state determination can be performed by volunteers and it can be immediately useful to them. Several recommendations can be made with regard to the use of trophic state classifications in volunteer programs.

1. Use the simplest definition of trophic state: neither the coordinator nor the volunteer will benefit by making the concept complex or somehow mysterious.
2. The recommended definition is plant biomass: it is historically correct, simple to measure, and simple for a volunteer (or manager) to understand. It also can be predicted from nutrient models and can be used to predict other biological characteristics.
3. Remove the mystery from the term eutrophication. Rather than linking the process to nutrients, which can cause all sorts of interpretational problems, simply define it as a movement of the lake's trophic state in the direction of more plant biomass. The definition is simple and more functional than other definitions.
4. The trophic state index of Carlson (1977) is recommended as the simplest method of relating trophic state concepts to volunteers.
5. If data for chlorophyll and phosphorus are available, use chlorophyll as the primary index for trophic state classification. Use the deviations of the Secchi depth and total phosphorus indices from the chlorophyll index to infer additional information about lake function.
6. Use the index as a teaching tool. Discuss with the volunteers all the possible factors, not just nutrients, that could make a lake more eutrophic. For example, explain that the deposition of erosional materials will cause the lake to become shallower and enhance macrophyte growth, thus affecting the total amount of biomass. Discuss the ramifications of change in plant biomass, how it affects hypolimnetic oxygen and fish species and its possible effect on food chains and recreational potential.
7. Be very careful about using quality terms when speaking of trophic state. Even your own perception of quality is affected by your background and education. Be sensitive to the fact that not all users will want the same type of water quality as you. Not everyone considers the ideal lake to be clear. Always be sensitive to the background and needs of the users.

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