1997-98 WISCONSIN DNR LAKE MANAGEMENT PLANNING GRANT FINAL REPORT FOR LAKES AMNICON AND DOWLING IN DOUGLAS COUNTY

SUBMITTED TO THE AMNICON AND DOWLING LAKES MANAGEMENT AND SANITARY DISTRICT

FROM C.J. OWEN AND ASSOCIATES MARCH 1998

Introduction and Grant Criteria

The following project summary is presented by C.J. Owen and Associates to the Amnicon and Dowling Lakes Management and Sanitary District. The project was funded by a 1997-1998 Wisconsin DNR Lake Management Planning Grant. The planning grants are intended to provide funding for the lake management *planning* process. Up to \$10,000 per project and a maximum of \$50,000 is available at a 75% state cost share. The intent of the grant program is to help *develop* comprehensive management plans depending on the condition and needs of the lake (which the planning process will help determine). The plan will specify activities related to minimizing the impacts of future development, managing user conflicts, and improving fishing and water quality.

The current project focuses on developing long term, resident staffed, management activities to help monitor and maintain the health of the lake. In an effort to address this

focus, analysis of water quality parameters have considered the whole lake as a potentially impacted resource, influenced by the entire community within the lake(s) watershed. Results from the lake studies of 1994, 1995, 1996 and this report should be characterized as an <u>overall</u> community concern. It should not be interpreted as indicating individual(s) responsibilities for a source of impact from a given geographical area. Resources are not available to assess each individual on quantifiable impacts to the water quality of the lakes, rather the data suggests possible avenues by which the whole community may be impacting the water resource.

The intent of this project is to develop a holistic approach to watershed management and should not focus on one perceived problem area. This is the case for the current and previous planning grant projects in that, although of concern to the district members, the focus should not be placed solely on septic issues. Rather all watershed pollution issues should be considered in the context of the continuation of the long term monitoring of water quality in Lakes Amnicon and Dowling.

When using the data generated by these studies it should be recognized that the focus of the sampling design is on protection of the health of the lake (i.e. the processes that effect how rapidly the lake ages (eutrophication) and the "usability" of the water resource by humans). Some of the data gathered may also be taken into consideration when analyzing possible impacts to the health and well being of the people utilizing the lakes.

Monitoring-Field

All in-lake monitoring for this project was carried out between July 19, 1997 and October 17, 1997 and will continue at ice out in the spring of 1998. Monitoring and sampling was conducted by residents of the Lake Management District under the training and supervision of C.J. Owen and Associates.

Study Lakes

Lake Amnicon is located in west central Douglas County, Wisconsin. Amnicon is a 172.4 hectare, relatively shallow (maximum depth 9.5 meters; mean depth 3 meter) drainage lake of glacial origin. Shoreline distance (not including two state owned islands) is 8.22 kilometers. Lake volume is approximately 5,200,000 cubic meters. The trophic status of Lake Amnicon is eutrophic (1993, 1994, 1995 and 1996 final reports by C.J. Owen and Associates). The shoreline of Amnicon is characterized as heavily developed, containing 2 and 3 tier development. One hundred and forty-eight shoreline lots were observed in 1994, resulting in a ratio of twenty-nine lots per shoreline mile. There are three (3) public boat launches and one campground on the lake.

Dowling Lake is adjacent to Amnicon Lake in west central Douglas County, Wisconsin. Dowling is a 62.24 hectare, shallow (maximum depth of 3.96 meters; mean depth 2.13 meters), drainage lake. Dowling is the major inflow to Amnicon Lake. Shoreline distance (not including islands) is 3.14 kilometers. The lake volume is approximately 1,400,000 cubic meters. The trophic status of Lake Dowling is eutrophic (1993,1994,1995 and 1996 reports by C.J. Owen and Associates). The shoreline of Dowling is characterized as heavily developed, containing 2 and 3 tier development. Eighty-two shoreline lots were observed in 1994 resulting in 41 lots per shoreline mile. There is one (1) public boat launch and no campgrounds on Dowling. (Appendix 1: Map 1 and 2).

Sampling Methodology

Water temperature, dissolved oxygen concentration and specific conductivity at each sampling site were measured at one meter intervals. Secchi disk transparency was also recorded at each site. Samples of the epilimnion (shallow sample), collected for chemical and biological analysis, were sampled 0.2 meter below the surface. In addition to surface samples, maximum depth samples were also collected using a Van Dorn sampler. The deep samples were collected 1 meter off the bottom (the bottom of the hypolimnion).

Field Methods-Chemical Samples

Field measurement and sample collection were carried out using routine limnological practices (Kallar et al. 1981; Lind 1985; EPA 1989). Temperature, dissolved oxygen, and conductivity were measured with a YSI 85 probe. All probes were calibrated prior to each sampling. In the field, probes were lowered to the designated depth and allowed to equilibrate before measurements were recorded. Profiles were taken monthly from July 19, 1997 until October 17, 1997 and will continue at ice out in the spring of 1998. Water transparency for the lake was measured using a standard 20 cm diameter Secchi disk.

Discrete samples were collected with a one-liter PVC Van Dorn water sampler. Samples were placed in pre-labeled polyethylene bottles containing the sampling date, lake name, site number and depth (provided by the State Laboratory of Hygiene). Samples were preserved according to instructions provided by the State Laboratory of Hygiene and placed into coolers, kept dark and iced during transport to the laboratory by overnight express UPS.

Project Description

In May of 1997 the commissioners of the Amnicon/Dowling Lake Management and Sanitary District contracted with the local water quality consulting firm of C.J. Owen and Associates to assist them with the following 1997-1998 Wisconsin DNR Lake Management Planning Grant project. To foster greater lake stewardship, the district agreed to take responsibility for the monthly physical and chemical monitoring of the two lakes, with C.J. Owen and Associates providing sample collection and processing training and data review. This recognized need for citizen input to the program was prompted by the districts continuing public education to the community and the public's awareness of the many issues that may be impacting the current and future water quality of the lakes. It was recognized that the greatest threat to the current lake water quality monitoring program was the loss of "momentum". The historical review of the lake data identified several past efforts by the district to obtain information on the water quality of the lakes, only to result in large gaps in data as enthusiasm in the lake monitoring programs of the 1970s and early 1980s decreased. The district feels that a large cause of this gap in data was a result of the increased reliance on consultants to perform standard water quality sampling and as a result prompted a loss in lakeshore property owner's "connection" to the program. This loss in momentum (and resulting gaps in data) was a critical missing piece in the discussion process related to current water quality issues being discussed by the district. As such a project with the following goals and objectives was undertaken to obtain the tools and instruction to take lake district ownership of the lake monitoring program.

Objectives

Objective 1: Obtain funding to equip district members with the basic analysis and collection equipment to allow for continued gathering of physical/chemical/biological samples and for obtaining dissolved oxygen, temperature and conductivity profiles of the study lakes.

Objective 2: Train 6-12 individuals (3-6 per lake) on the correct sample collection and processing techniques and the use of field analytical tools for sampling resources related to in-lake water quality. Training should emphasize physical and chemical water sampling, obtaining bacteriological, phytoplankton and well water samples and determining the identity of beneficial and unwanted macrophyte species.

Objective 3: Continue to provide onsite training to property owners in "groundtruthing" of the 1994 Aerial Lakeshore Analysis. Continue to instruct individuals on how to recognize areas that can be improved by implementation of lakeshore "Best Management Practices" (BMPs) as a resource to other property owners.

Objective 4: Continue the chemical, biological and physical characterization of Lakes Amnicon and Dowling, thereby maintaining a coherent lakes data set that can be used by the district in future lake management discussions. This can be accomplished by obtaining monthly oxygen, conductivity and temperature profiles and evaluating transparency changes by monitoring secchi depth readings. Chemical analysis of the two lakes will consist of Chlorophyll-a, NO₃, NH₄, TP and Ortho-P from surface and bottom samples at the deep hole locations taken on a monthly basis.

Results

Objective 1: The district has been supplied with the following analytical equipment to provide accurate and consistent limnological data acquisition.

- * One YSI Model 85 Handheld oxygen, conductivity, salinity, and temperature meter. (See Appendix 2 for description and operations manual).
- * One 20 cm Secchi Disk with metered line.

- * One VanDorn water sampling device with metered line and trigger messenger.
- * One 200 mm diameter plankton tow net.
- * One rigid plastic carrying case for above equipment.
- * One Gelman 4.5 cm magnetic filter tower with sidearm flask, pressure pump, one 1 liter graduated cylinder and misc. GFC and .45 m filters.

This equipment is available to interested district members at the discretion of the district commissioners and only after the completion of adequate training in its care and use.

Objective 2: Five (5) one half day training sessions were provided to interested district members on the correct procedures for sample collection and the operation of the diagnostic equipment. These training sessions were conducted by C.J. Owen and Associates in accordance with Wisconsin DNR, Wisconsin State Laboratory of Hygiene, and U.S. EPA guidelines. Training consisted of three sessions involving in-field hands-on demonstrations of correct field sampling protocol and documentation, analytical equipment operation, and concepts and procedures that will help provide less human impact to the lake systems. These three training sessions were facilitated by a training manual that was provided to participants and the district as a permanent record of collection techniques (see Appendix 3).

A fourth training session was devoted to the in-field recognition and identification of various macrophyte species within the two lake systems. Cindy Hagley, a macrophyte specialist with Minnesota Sea Grant, provided training and identification information on the common plant species that were identified in the earlier (1995) macrophyte assay of the two lakes. Exotic species identification and control guidelines were also a part of this training session. A document entitled [Aquatic Plants of Lakes Amnicon and Dowling, Wisconsin]] was compiled and provided to the district and participants of this session. A copy of this document is included in Appendix 4.

The fifth training session consisted of 1997 summer data review, reporting, and basic limnological analysis. It has been determined by the district that continued limnological sampling and trend analysis of the two lakes will be provided through a partnership with the University of Wisconsin - Superior. This final session involved an explanation of the data compiled over the previous year's studies (1993-1996) and recommendations for future analysis.

Objective 3: As part of the 1997 planning grant, A.W. Research Laboratories sent a professional groundtruthing specialist to continue work with the interested district members. A final report on the results of the project is provided in Appendix 5.

Objective 4: Adequately trained members of the district were responsible for the collection, processing and shipment of the various lake samples to the Wisconsin State Laboratory of Hygiene. The type and number of chemical analytes measured are outlined

in Table 1 with year to date results noted in Tables 2 & 3. It was determined that sample collection would be suspended in the fall of 1997 and resumed in the spring of 1998 for best utilization of the district's resources. Water temperature, dissolved oxygen concentration and specific conductivity at each sampling site were measured at one meter intervals. Secchi disk transparency was also recorded at each site. This data is recorded in Tables 4 thru 9.

Analytes	July	Aug	Sept	April	May	June	July	<u>Total</u>
Chlorophyll-a*	1	1	1	1	1	1	1	7
Nitrate+Nitrite	2	2	2	2	2	2	2	14
Ammonia-N	2	2	2	2	2	2	2	14
Total-P	2	2	2	2	2	2	2	14
Ortho-P	2	2	2	2	2	2	2	14
Chloride	2			2		2		6
Color	1			1		1		3
Calcium	2			2		2		6
Iron	2			2		2		6
Magnesium	2			2		2		6
Manganese	2			2		2		6
Potassium	2			2		2		6
Sodium	2			2		2		6

Table 1. Amnicon/Dowling-Epilimnetic and Hypolimnetic Per Lake Chemical Sampling Schedule at Historic Deep Hole Sites (*Chlorophyll-a is sampled from surface only).

Sample Date	Chl-a (ug/l)	NO3 (mg/l)	NH3 (mg/l)	TP (mg/l)	OP (mg/l)	Chlo (mg/l)	Color	Ca (mg/l)	Fe (mg/l)	Mg (mg/l)	Mn (ug/l)	K (mg/l)	Na (mg/l)
Amnicon Epilimnion													
7/19/1997	5.68	ND	ND	.019	.002			6.2	0.10	2.3	0.4	0.6	2.4
8/09/1997	3.47	ND	.009	.017	.001								
9/08/1997	11.0	.01	.013	.024	.002								
Amnicon Hypolimnion													
7/19/1997		ND	ND	.032	.003	2.4	5 Q	7.0	0.18	2.5	380	0.6	2.5
8/09/1997		ND	.60	.032	.009								
9/08/1997		.015	.053	.027	.002								

 Table 2. Amnicon Epilimnetic and Hypolimnetic Per Lake Chemical Sampling

 Results at Historic Deep Hole Sites (*Chlorophyll-a is sampled from surface only).

Sample Date	Chl-a (ug/l)	NO3 (mg/l)	NH3 (mg/l)	TP (mg/l)	OP (mg/l)	Chio (mg/l)	Color	Ca (mg/l)	Fe (mg/l)	Mg (mg/l)	Mn (ug/l)	K (mg/l)	Na (mg/l)
Dowling Epilimnion													
7/20/1997 8/09/1997 9/08/1997	8.62 10.3 12.7	ND ND .01	ND .002 .013	.035 .033 .040	.005 .001 .002	4.4	70	5.4	0.66	2.1	28	0.6	1.5
Dowling Hypolimnion													
7/20/1997 8/09/1997 9/08/1997		.010 ND .020	.019 .01 .056	.055 .036 .022	.014 .002 .002	1.4		6.0	0.92	2.2	390	0.7	2.0

 Table 3. Dowling Epilimnetic and Hypolimnetic Per Lake Chemical Sampling

 Results at Historic Deep Hole Sites (*Chlorophyll-a is sampled from surface only).

AMNICON CONDUCTIVITY & SECCHI

Date	7/19/1997	8/09/1997	9/06/1997	10/17/1997	
Secchi	2.10	2.0	1.8	2.5	
Surface	61.5	59.0	57.4	45.6	
1 meter	61.5	60.9	57.4	45.8	
2 meter	60.8	60.8	57.4	45.5	
3 meter	61.0	60.7	57.3	45.6	
4 meter	58.0	60.7	57.1	45.6	
5 meter	57.2	60.7	57.4	45.5	
6 meter		83.6	57.6	45.5	
7 meter		96.6		45.6	
Bottom	71.0	104.7	64.1	45.6	

 Table 4. Amnicon 1997 Conductivity and Secchi Measurements at Historic Deep

 Hole Sites. Secchi is measured in meters and conductivity in umhos.

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AMNICON DISSOLVED OXYGEN

Date	7/19/1997	8/09/1997	9/06/1997	10/17/1997
Surface	6.81	7.7	8.59	9.7
1 meter	6.58	7.50	8.54	10.1
2 meter	6.86	7.59	8.47	9.8
3 meter	6.10	7.50	8.36	9.8
4 meter	3.32	7.62	7.90	9.9
5 meter	0.70	7.60	6.44	9.6
6 meter		0.03	5.85	9.7
7 meter		0		4.8
Bottom	0.0	0	0.07	3.4

Table 5. Amnicon 1997 Dissolved Oxygen Measurements at Historic Deep Hole Sites. D.O. is measured in mg/l.

AMNICON <u>TEMPERATURE</u>

7/19/1997	8/09/1997	9/06/1997	10/17/1997
24.3	23.1	20.2	11.5
24.4	23.1	20.1	11.4
24.4	23.1	20.0	11.3
24.3	23.0	20.0	11.2
21.0	23.0	19.7	11.2
18.4	23.0	19.2	11.2
	16.9	19.0	11.2
	14.1		11.2
15.9	12.9	17.1	11.2
	7/19/1997 24.3 24.4 24.4 24.3 21.0 18.4	7/19/19978/09/199724.323.124.423.124.423.124.323.021.023.018.423.016.914.115.912.9	7/19/1997 8/09/1997 9/06/1997 24.3 23.1 20.2 24.4 23.1 20.1 24.4 23.1 20.0 24.3 23.0 20.0 21.0 23.0 19.7 18.4 23.0 19.2 16.9 19.0 14.1 15.9 12.9

 Table 6. Amnicon 1997 Temperature Measurements at Historic Deep Hole Sites.

 Measured in degrees centigrade.

DOWLING CONDUCTIVITY & SECCHI

Date	7/20/1997	8/09/1997	9/06/1997	10/17/1997	
Secchi	1.5	1.62	1.5	1.5	
Surface	48.5	50.1	46.6		
1 meter	48.2	50.1	46.5		
2 meter	47.8		46.5		
3 meter	48.3		46.5		
Bottom	56.9	49.9	46.5		

 Table 7. Dowling 1997 Conductivity and Secchi Measurements at Historic Deep

 Hole Sites. Secchi is measured in meters and conductivity in umhos.

DOWLING DISSOLVED OXYGEN

Date	7/20/1997	8/09/1997	9/06/1997	10/17/1997
Surface	5.25	7.8	8.14	9.8
1 meter	4.4	7.8	8.08	9.7
2 meter	4.7		7.99	9.7
3 meter	.73			9.6
Bottom	0	7.7	7.56	9.2

 Table 8. Dowling 1997 Dissolved Oxygen Measurements at Historic Deep Hole Sites.

 D.O. is measured in mg/l.

DOWLING <u>TEMPERATURE</u>

Date	7/20/1997	8/09/1997	9/06/1997	10/17/1997
Surface	23.7	23.0	19.9	11.1
1 meter	23.7	22.9	19.8	10.9
2 meter	23.4		1 9.8	10.8
3 meter	21.0			10.7
Bottom	18.7	22.9	19.6	10. 6

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Table 9. Dowling 1997 Temperature Measurements at Historic Deep Hole Sites.Temperature is measured in degrees centigrade.

Appendix 1





1997 AMNICON/DOWLING LAKE MANAGEMENT & LIMNOLOGICAL PRIMER



Solutions for a Better Environment C.J. Owen & Associates Progressive Systems International Inc.

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INTRODUCTION

It is commonly recognized that "bad" data is worse than no data at all. Not only are your time and money wasted, but environmental management decisions may be made which create or worsen a problem simply because those decisions were based on worthless information. For data to be useful, the quality must be adequate and known.

Knowing the quality of data is as important as the data itself. This document will present quality control guidelines for the development of a monitoring program capable of providing accurate lake management data. Each monitoring group should develop their own quality assurance plan for each project before monitoring begins. This will enable anyone in the future, unfamiliar with the project, to repeat exactly the procedures associated with the data collected for that project. With this consistency, comparisons over long periods of time, perhaps decades, can be made. Documented methods of how, when and where samples were taken erases any doubt as to their validity. <u>Word of mouth and memory are not sufficient</u>. **DETAILED DOCUMENTATION** will allow future evaluation of data quality. If the procedures used in the past can be reproduced, proper interpretations of historic data can be made.

Before monitoring begins, decide what your program is trying to accomplish and what your budget will allow. Many monitoring programs are designed to characterize long term trends in water quality as well as determination of current trophic status with the calculation a trophic state index (TSI).

TROPHIC STATUS

Since the early part of the 20th century, lakes have been classified according to their *trophic state*. "Trophic" means nutrition or growth. A *eutrophic ("well* nourished") lake has high nutrients and high plant growth. An *oligotrophic lake* has low nutrient concentrations and thus low plant growth. In between, are *mesotrophic* lakes. The trophic status of a lake is affected by the age and shape of the lake, geology of the watershed, ratio of watershed area to lake area, flushing rate of water through the lake, human impact, and many other factors. Consequently, while lakes may be lumped into a few trophic classes, each lake has a unique constellation of attributes that contribute to its trophic status.

Trophic status is a useful means of classifying lakes and describing lake processes. Oligotrophic versus eutrophic were terms originally used to distinguish highland versus lowland lakes in Germany. Highlands and other areas with infertile soils release comparatively little nitrogen and phosphorus to lakes. In contrast, lowland areas with rich organic soils release larger amounts of nutrients into nearby waters.

In addition to describing geographic variation, the "trophic" terms are used to describe geologic as well as recent history of lakes. *Eutrophication*, the progress of a lake toward a eutrophic condition, is often discussed in terms of a lakes history. A typical lake is said to age from a young, oligotrophic lake to an older, eutrophic lake (Figure 1). Turbulent geological events, such as glaciation, created lakes in uneven land surfaces and depressions. The landscapes surrounding lakes were often infertile and thus many lakes were oligotrophic. Eventually some of the areas supported colonizing organisms that decomposed rough cover materials into reasonably fertile soils. Active biological communities developed and lake basins became shallower and more eutrophic as decaying plant and animal material accumulated on the bottom. There are undoubtedly exceptions to this typical historical development where geology, topography, and lake morphology caused eutrophic conditions from the start.

This concept of lake aging has unfortunately been interpreted by some as an irreversible process whereby a lake eventually dies. In the 1960s this was an issue exemplified by the hypereutrophic condition of Lake Erie. Although it was pronounced dead, it eventually returned to less eutrophic conditions when major point sources of phosphorus (i.e., wastewater treatment plants) were controlled in the early 1970s.



Figure 1.

Furthermore, research in paleolimnology has provided some evidence that contradicts the idealized version of a lake aging as continued eutrophication. Studies of sediment cores have suggested that the algal productivity of Wisconsin lakes actually may have fluctuated a great deal during the past 12 to 14 thousand years (the period since the last glaciation). Changes in climate and watershed vegetation seem to have both increased and decreased lake productivity over this period. It is probable that some lakes experienced high rates of photosynthesis fairly soon after glacial retreat and then became less productive until recent times. It is also possible that water sources for some lakes have changed over the past thousands of years through diversions of stream flow. In such cases water supplies to a lake (and therefore nutrient supplies) could have changed, and the lake's productivity may have changed also.

TROPHIC STATE INDICES

Trophic state indices (TSIs) are an endeavor to provide a single quantitative index for the purpose of classifying and ranking lakes, most often from the standpoint of evaluating water quality. In recent years, the Carlson (1977) Index appears to have attained general acceptance in the limnological community as a rational approach to this classification problem. A number of modifications and regional "customizations"

of his approach have occurred, but for this evaluation Carlson's index will be used because of its historical use by the Wisconsin-DNR in past evaluations.

Carlson's index results in values ranging from 0 to 100 with increasing values indicating more eutrophic conditions (Table 1). The trophic states for the index are defined by using each doubling of Secchi transparency as the criterion for the division between each state, i.e. each time the transparency doubles from some base value, a decrease in TSI-S (trophic state indices for Secchi depth) occurs. The relation of Secchi depth to total phosphorus is a simple inverse function, so a doubling of total phosphorus causes the TSI-P (trophic state indices for phosphorus) to increase by 10 units. Both TSI-P and TSI-S are related to chlorophyll-<u>a</u> concentration. The resulting relationship results in the third TSI, TSI-C (trophic state indices for chlorophyll). The indices are based on the following three expressions:

TSI-P= $4.15 + (14.42 * \ln TP)$, in $\mu g/L$.

TSI-C= $30.6 + (9.81 * \ln \text{Chlorophyll-a})$, in $\mu g/L$.

TSI-S= 60.0 - (14.41 * ln Secchi Depth), in meters.

Carlson's Tropbic State Index

TSI <30	Classical oligotrophy: Clear water, oxygen throughout the year in hypolimnion, salmonid fisheries in deep lakes.
TSI 30-40	Deeper lakes still exhibiting classical oligotrophy, but some shallower lakes will become anoxic in the hypolimnion during the summer.
TSI 40-50	Water moderately clear, but increasing probability of anoxia in hypolimnion during summer.
TSI 50-60	Lower boundary of classical eutrophy: Decreased transparency, anoxic hypolinmia during summer.
TSI 60-70	Dominance of blue-green algae, algal scums probable, extensive macrophyte problems.
TSI 70-80	Heavy algal blooms possible throughout the <u>summer</u> , dense macrophyte beds, but extent limited by light penetration. Often would be classified as hypereutrophic.
TSI >80	Algal scums, summer fish kills, few macrophytes, an over abundance of rough fish.

Table 1.

The following "rules" should be applied when using TSIs:

- 1) If one index value is based on numerous measurements, while the other is based on a single measurement, then the former is used as a better indicator.
- 2) If there is only a single measure for each or an equal number of measures, the TSI-P value should be favored.
- 3) TSI-S and TSI-C based on a single measure are to be viewed with caution.

STANDARD FIELD OPERATING PROCEDURES.

Monitoring regimes range from simple and inexpensive (obtaining Secchi disk transparency readings every other week from May to September) to intensive and expensive (biweekly transparencies, temperature/ dissolved oxygen profiles, total phosphorus, chlorophyll, and other chemical data may be obtained). Usually, the monitoring regime which best suits the budget and goals of the monitoring program falls somewhere between these two extremes. It is best to consult with a Wisconsin DNR lakes biologist for advice on the design of your program.

This manual is intended for use by volunteer monitors but also documents the common methods used by lake biologists. Nothing in this manual replaces the guidance offered by equipment manufacturers concerning care and calibration of their products.

The Standard Field Operating Procedures that follow, outline steps used to collect and record lake data. If samples are not taken the same way each time, data will not be comparable, nor will the procedures used to collect data be repeatable in the future. It is absolutely necessary to locate the same sample locations and repeat the procedures used to collect water quality data in order to use the data in the future.

ESTABLISHING SAMPLE LOCATIONS FOR LAKES

- 1. Obtain a Bathymetric map of the lake to be sampled (Figures 2&3).
- 2. Look for the deepest recorded depth on the map. Circle the area with the deepest readings (from now on referred to as the "deep hole"). This area will be the primary sampling station (location) for the lake and should be called Station # 1. The deep hole is usually Station # 1. Most lakes only require one sampling location, however, if more than one station is desired, the area with the second deepest area is Station # 2 and so on. Sometimes a lake will have two deep holes at different places in the lake. In this case, the hole that appears to cover the largest area should be labeled Station # 1. Sample stations are not the same as lake basins and should not be used as such. If a different protocol is preferred for a project, document the change, otherwise the protocol described above will be assumed.

NOTE: It is best to check with the Wisconsin DNR prior to establishing a sampling location to obtain the lake's identification statistics and verify that the appropriate station number is assigned.

If a DNR map is not available (lake has never been surveyed by DNR), photocopy the lake from a map and mark the location to be sampled. It may take some actual field depth readings with a depth finder or weight

on a measured rope to find a spot deep enough to collect the appropriate analytes. Speaking with long time residents may aid in locating the deepest hole. Sometimes if shoreline slope is very steep in a particular area, the greatest depths will be found in adjacent offshore waters.



Figure 2.

- 3. <u>Always anchor</u> at the same place each time a station is sampled. Drifting, even on calm days, can result in inaccurate depth estimates and thus, inaccurate samples.
- 4. It is very important to document as closely as possible the location that is being sampled. Remember, future samplers who never come in contact with you, may be trying to sample the same area. Record land features which will help locate the station on your map. Reference these features such that the sampling location is at the mid point of an X (e.g., station is located at the intersection of the line drawn between Duck Island (east) and Foster Point (west) and the line drawn between massive rock on north shore and red house with green trim on the south shore).





FILLING OUT FIELD FORMS

Filling out forms properly is extremely important. Field forms describe the sample location as well as methods used and results of tests performed in the field. Inadequate sampling location identifiers could result in analysis being entered for another location or not entered at all. Whichever form is used, make sure it has space to properly identify sample location, methods used in the field and results obtained. Other information that may be critical includes weather conditions and remarks that may further qualify the results obtained or are of interest to the program.

A standard field form is shown in Appendix 1.

SAMPLING PROTOCOL

WATER COLLECTION TECHNIQUES

Two types of water samples are routinely collected: deep samples and surface grabs. Each will be discussed separately. Regardless of which is collected, be sure there is no gas/oil sheen or other obvious contaminants on the water surface where samples are to be collected.

SURFACE GRAB SAMPLES

Grab samples are most often used to obtain water samples at or just below the water/air interface where more complicated sampling devices are unnecessary.

SURFACE GRAB SAMPLE PROCEDURE

- 1. Label all field sample containers before they come in contact with the water. The label should include lake name, station #, depth, type (G=grab), date and initials. Indelible ink is preferred because some inks will bleed.
- 2. Rinse the field sample container and cap with water from the collection site to remove any impurities.
- 3. Surface grab samples can be obtained simply by inverting the field sampling container, submerging it to a depth of approximately 6 inches then allowing the container to fill. The inversion minimizes contamination from debris floating on the water surface.
- 4. Fill the field sampling container to over flowing removing as much air as possible
- 5. Replace cap using care not to touch inside of cap. Store on ice and refrigerate as soon as possible.
- 6. Record the depth the sample is taken at and any other relevant information (such as water temperature) on the field form.

DEEP WATER SAMPLES

Samples from deeper levels require the use of one of a number of "discrete depth samplers". Kemmerer samplers or Van Dorn samplers are used to obtain deep samples. Any approved sampling device that might be used for water quality testing should obtain a representative sample. Rope or chain used to lower sampling device into water should be marked in 1 meter intervals with the mid point of the device as the zero reference.



Regardless of what device is used, care must be taken to keep it clean and free of contaminants and sediments. The same basic steps are used to obtain the deep water samples:

- 1. Label field sample containers.
- 2. Inspect the sampling device and set the tripping mechanism.
- 3. Lower device slowly to the desired depth.
- 4. Raise and lower the device about 8-12 inches around the desired depth at least 5 times to assure that any water trapped while lowering the device has been replaced with water from the desired depth.

NOTE: If taking grab samples just above the sediment surface, or at the epi-metalimnion or metahypolimnion interface, it is extremely important to avoid contamination from sediment or mixing of water layers. In these cases, when device is 2 meters above desired depth, lower <u>very slowly</u> to desired depth.

- 5. Trip the device and raise it gently to the surface.
- 6. Take care to avoid touching the end of the device from which the samples are removed to avoid contamination.
- 7. Decant water into the field sample container. Rinse and discard. Fill field sample container with desired amount taking care to remove as much air as possible.
- 8. Replace cap using care not to touch inside of cap. Store on ice and refrigerate as soon as possible.
- 9. Record the depth and type of samples on the field form

QUALITY ASSURANCE FOR SAMPLES

For every 10 samples taken, a duplicate sample should be taken and labeled as such. A duplicate sample is taken from the same water sample as the first. If results are radically different (i.e. one result more than 10% greater than the other), the results should be questioned. Check the laboratory, sampling method and handling for sources of contamination. Repeat duplicate process next time sample(s) are taken. If result in question cannot be explained, do not record results into the database.

SECCHI DISK TRANSPARENCY

What is a "Secchi?"

Father Pietro Angelo Secchi, scientific advisor to the Pope, was asked by Commander Cialdi, head of the Papal Navy, to test a new transparency instrument. This instrument, now named the "Secchi" (rhymes with "checky") disk, was first lowered from the papal steam yacht, l'Immacolata Concezione (The Immaculate Conception) in the Mediterranean Sea on April 20, 1865. Secchi (1818-1878) was actually a famous astronomer, one of the first astrophysicists. A pioneer in the application of photography to astronomy (he photographed an eclipse of the sun in 1851). He was probably lucky that he did not gain recognition for his color map of Mars on which he labeled faint tracings as canali, the Italian word for "channels." Another Italian, Giovanni Schiaparelli, expanded the number and legibility of the canali on his map. Later these canali, or channels, were misinterpreted to mean artificial canals, leading to speculation of life on Mars. In a sense, Secchi was the progenitor of some great (and not so great) science fiction.



Father Pietro Angelo Secchi

The Secchi disk measures the transparency of water. Transparency can be affected by the color of the water, algae, and suspended sediments. Transparency decreases as color, suspended sediments, or algal abundance increases.

Water is often stained yellow or brown by decaying plant matter. In bogs and some lakes the brown stain can make the water the color of strong tea. Algae are small, green aquatic plants whose abundance is related to the amount of plant nutrients, especially phosphorus and nitrogen. Transparency can therefore be affected by the amount of plant nutrients coming into the lake from sources such as sewage treatment plants, septic tanks, and lawn and agricultural fertilizer. Suspended sediments often come from sources such as resuspension from the lake bottom, construction sites, agricultural fields, and urban storm runoff.

Transparency is an indicator of the impact of human activity on the land surrounding the lake. If transparency is measured through the season and from year to year, trends in transparency may be observed. Transparency can serve as an early warning that activities on the land are having an effect on a lake.

A Secchi disk is a weighted circular plate, 8 inches in diameter with opposing black and white quarters painted on the surface and black painted on the bottom (Figure 4). It is used to determine the clarity or transparency of the water. It is attached to a low stretch line or chain that has been marked with single marks at ½ meter intervals and double marks for each full meter. When held by the line the disk should hang horizontally.

The Secchi disk works as a contrast instrument. It disappears when the human eye can no longer see it, meaning that there is no contrast between the disk and its background. The black quadrants may serve as a constant black background, thus standardizing the contrast. Most limnological disks now use the black and white disk while marine disks remain all white. The black and white quadrants were apparently the invention of George Chandler Whipple, a professor of Sanitary Engineering at Harvard University. He mentions the modification in a 1899 book, The Microscopy of Drinking Water.

Diagram of Secchi Disk



Figure 4.

Take the Secchi disk reading on the shady side of the boat (sun on your back). Secchi disk transparencies should be determined between the hours of 9:00 AM (0900 hours) and 3:00 PM (1500 hours). Wind, waves, as well as brightness of sun will influence the depth of the Secchi disk reading, therefore, it is necessary to note these critical weather conditions on the field sheet along with the time of day the reading was taken. Secchi transparency readings should be done every other week from May through October.

SECCHI DISK PROCEDURE

- 1. Anchor the boat. It is very important that the Secchi disk hangs straight up and down in the water column. If the boat is drifting from the wind, an inaccurate measurement will result. An inadequately weighted Secchi disk will not hang straight up and down in the water. If this happens, do not take a reading until more weight is added to the disk.
- 2. Lower the disk over the shaded side of the boat. Do not wear sunglasses while obtaining readings. If photo gray prescription glasses are worn, place them in the shade long enough for them to clear.
- 3. Watch the Secchi disk as it is slowly lowered until it totally disappears from view (including glow).
- 4. Record this level by pinching with fingers or putting a clothespin on the line or chain at the water surface. Slowly raise the disk until it reappears. The second position of the line or chain (at the water surface) is recorded (pinch with fingers or put a clothespin on it) with the transparency reading being the midpoint between these two locations. In other words, the Secchi disk reading is the average or midpoint between the depth the disk disappears and the point at which it reappears. Estimate this depth to the nearest 1/10th (0.1) meter and record the depth, location, and other relevant information on your field sheet.

Secchi disk parameters are recorded to 1/10th meter (1 decimal place to the right of decimal point). If it is necessary to measure transparency in feet, estimate depth to the nearest 0.5 ft. (6 inches). Record units used on the field sheet.

QUALITY CONTROL FOR SECCHI DISK READINGS

Take duplicate readings every 10th sample. If more than one person obtains readings, compare Secchi disk results at least once during the sampling season.

TEMPERATURE

A key factor in the physical structure of lakes is the unique character of water. Ice floats, while water with temperatures just above freezing sinks. We learned in our high school science class that as compounds go from a liquid to a solid the molecules are packed more tightly and consequently the compound is more dense as a solid than as a liquid. Water, in contrast, is most dense at 4°C and becomes less dense at both higher and lower temperatures. The density/temperature relationship of fresh water is shown in Figure 5.



Figure 5.

Because of this behavior of water, many lakes in temperate climates tend to stratify, or form layers, especially during the summer. In the spring, just before a lake's ice cover melts, the water near the bottom will be at 4°C. Water above that will be cooler, approaching O°C just under the ice. As the weather warms, the ice melts, and the surface water heats up (increases in density). Soon the temperature of the entire lake is 4°C. When the temperature (density) of the surface water equals the bottom water, very little wind energy is needed to mix the lake completely. After this spring "turnover," the sun shines more intensely, the surface water absorbs heat and becomes warmer. For a while winds may still mix the lake from bottom to top, but eventually the upper water becomes warm and too light to mix completely with

dense, deeper water. As Figure 5. suggests, the relatively large differences in density at high temperatures are very effective at preventing lake mixing.

As summer progresses, the temperature (and density) difference between upper and lower water becomes more distinct. Deep lakes generally become physically stratified into three identifiable layers, known as the *epilimnion, meta-limnion, and hypo-limnion* (Figure 6). The *epilimnion* is the upper, warm layer, and is typically well-mixed. Below the epilimnion is *the metalimnion or thermocline* region, a layer of water in which the temperature declines rapidly. The *hypolimnion* is the bottom layer of colder water, isolated from the epilimnion by the metalimnion. The physical barrier of the metalimnion's density change prevents mixing of upper and lower waters for several months of the summer.



Figure 6.

As the weather cools during autumn, the epilimnion cools also, reducing the density difference between it and the hypolimnion (Figure 7). As time passes, winds mix the lake to greater depths, and the thermocline gradually descends. When surface and bottom waters approach the same temperature (isothermal) and density, autumn winds can mix the entire lake and the lake is said to "turn over." As the atmosphere cools, the surface water continues to cool until frozen. Under the ice, there develops a less distinct density stratification with heavier warm 4°C water below cooler, lighter water.



Figure 7.

This pattern of spring turnover-summer stratification-fall turnover is typical for temperate lakes. Lakes with this pattern of two mixing periods are referred to as *dimictic*. Many shallow lakes, however, do not stratify in the summer, or stratify for only short periods throughout the summer. Lakes that stratify and destratify numerous times within a summer are known as *polymictic lakes*. Both polymictic and dimictic lakes are common in Wisconsin.



Figure 8.

Much less common are lakes that circulate incompletely because of a bottom water layer that remains stagnant. To distinguish them from the *holomictic (whole* mixing) lakes, these partially mixing lakes are referred to as *meromictic*. The nonmixing bottom layer is known as the *monimolimnion* and is separated from the overlying water (*mixolimnion*) by the *chemocline* (Figure 8). The stagnant, and typically anaerobic, monimolimnion has a high concentration of dissolved solids compared to the mixolimnion. In general, meromictic lakes have a large relative depth ratio of maximum depth to average diameter of lake surface.

Temperature is also important for several other reasons. Biological activity proceeds at a faster rate in warm water than in cold water, thus algal production, fish growth and biological decay all tend to be greater in the summer. Temperature also influences the kinds of plants and animals in the water.

PROCEDURE USING A STANDARD THERMOMETER

Using a thermometer, hold the thermometer just below the lake surface (6 inches) for at least 1 minute or until temperature reading is stable. Read the thermometer while it is still in the lake. Temperature can change rapidly on a hot day if the thermometer is removed from the lake to read the temperature. Take the temperature several times as a quality control procedure to ensure that precision is being achieved. Check the temperature of the thermometer against a known standard, if possible, to ensure accuracy of the instrument. A laboratory should have a thermometer with known calibration.

Temperature of grab samples can also be taken this way. Insert thermometer into shaded sample and wait for the temperature to stabilize. Read temperature with thermometer still in the sample. Temperature should be taken first to ensure accurate reading. Thermometers should be rinsed in surface water before inserting in sample to avoid contamination. Thermometers should be kept clean for the same reason.

Generally most electronic analytical measuring devices for water (i.e. D.O. meters, conductivity probes) include a built-in temperature probe. These are often more accurate and much easier temperature measuring devices than the standard thermometer and should be used whenever possible. The following section describes their use in further detail.

QUALITY CONTROL FOR TEMPERATURE

Take temperature readings 3 times at the 10^{th} measurement. Temperature should not range more than plus or minus (+/-) 1 degree. If results exceed this amount, replace the thermometer. Thermometers should be checked against a standard thermometer at least once a year. Thermometers can be purchased from a scientific supply company. Often a laboratory or University will allow calibration of a thermometer at their facilities.

DISSOLVED OXYGEN (D.O.) PROFILES

Temperature and dissolved oxygen are important factors which influence the biological activity and diversity that occurs in a lake (Figure 9). Bottom waters in deep lakes are generally replenished with oxygen during periods of overturn in the spring and fall when water temperatures are uniform throughout the water column. Once these lakes stratify, oxygen in the bottom waters is not replenished until the next period of overturn. Oxygen is consumed by aquatic life including organisms which decompose plankton and organic matter that settle out of the epilimnion. The lack of oxygen kills fish and other aquatic life, and can contribute to internal loading of phosphorus from the sediments. The following guidelines should be followed for taking D.O. profiles. These data are used for assessing the quality of lakes and the potential for internal recycling of phosphorus.



Figure 9.

Temp/D.O. profiles can be taken at any time of the year, however the most useful information from these profiles is obtained during the period a lake is most stressed (July-September and February-March). Temp/D.O. readings should be taken at discrete intervals of one meter. In deep lakes (greater than 20 meters), time constraints may warrant a different protocol. For lakes greater than 20 m in depth, take Temp/D.O. readings every meter until through the entire epilimnion and metalimnion (usually 10-15 meters). Once into the hypolimnion, take readings at least every other meter.

DISSOLVED OXYGEN METERS

Several manufacturers make dissolved oxygen meters (Figure 10). Always follow manufacturer's directions for obtaining dissolved oxygen readings (Reference YSI Model 85 Handheld Oxygen, Conductivity, Salinity, and Temperature System Operations Manual). It is recommended that Temp/D.O. profiles be taken at the established sampling stations using a cable marked in 1 meter intervals. Regardless of which meter is used, always check batteries and membrane before going into the field and calibrate before immersing the probe in the lake.

STANDARD OPERATING PROCEDURES FOR D.O./TEMP PROBES

- 1. Follow manufacturer's instructions. Allow meter to warm up 15 to 20 minutes before calibration. Calibration should be done only when the meter has warmed up and the probe and meter are at the same temperature. If the meter and probe have been stored or exposed to different temperatures you may have to wait longer.
- 2. If the meter is equipped with a battery check, check batteries and replace if necessary.
- 3. If meter has the capability of a zero check do so with meter on. Adjust if necessary.

4. Remove the probe from the calibration chamber and make sure the sponge is damp. If sponge is dry, wet the sponge.



Figure 10.

- 5. Check the membrane for air bubbles and wrinkles. If bubbles or wrinkles are present, remove membrane and replace. Check to make sure no drops of water are clinging to the membrane. If drops are present, shake or blow to gently remove droplets. Don't tap; these probes are very fragile. Replace probe in the calibration chamber. NOTE: Bring extra membrane kits into the field in case a membrane is punctured/wrinkled or air bubbles are present.
- 6. Wait At least 5 minutes for air in the chamber to become saturated with water.
- 7. If a barometer is present, set meter to appropriate level of oxygen. If a barometer is not used, assume the appropriate level of oxygen for that temperature at 1 atmosphere (sea level or 760 mm hg). An oxygen/temperature chart is in the D.O. meter's owners manual. Photocopy and keep copy in carrying case with D.O. meter. Some meters such as the YSI 85 only require an estimate of elevation to calibrate for D.O.

- 8. Record the method used to determine D.O. on the field sheet (D.O. meter). To take readings, switch the dial to temperature mode, put the probe in the water and wait 10 to 20 seconds or until it stabilizes, record the temperature reading on field sheet then switch to the appropriate oxygen scale. Unless the probe is equipped with a stirrer, it is important to move the probe up and down steadily about 4 inches, at a rate of about 1 inch/second, to ensure a fresh supply of oxygen at the membrane surface. Moving the probe too slowly will underestimate D.O. levels. Moving the probe too fast will cause the needle to jump around and force oxygen into membrane resulting in a high, inaccurate reading. Wait for the system to stabilize (about 30 seconds, sometimes longer), record the oxygen level on the field sheet and switch back to the temperature scale.
- 9. Lower the probe to the next depth, repeat the sequence always recording the temperature first then D.O. readings. Gently raising and lowering of the probe at a rate of about 1 inch per second is extremely important for obtaining accurate dissolved oxygen readings as the probe is dependent on the amount of oxygen which passes across the membrane. If the profile looks unusual, bracket those readings and recheck on the way up. Note on the field sheet that those readings were rechecked.
- 10. During the course of a day, the meter should remain on between stations/lakes and the calibration should be checked before probe is submerged at the next site. Re-calibrate if necessary.

QUALITY CONTROL FOR D.O. METERS

D.O. accuracy is taken into consideration during the calibration process. However, for every 10th profile taken, 3 duplicate readings should be made randomly throughout that profile. Duplicate readings should not vary more than plus or minus 0.2 ppm. If readings vary more than 0.5 ppm, repair of membrane or meter is advised. Since temperature is critical to accurate D.O. readings, at least once a year the temperature of the D.O. meter should be compared to a thermometer with known calibration. Check the D.O. result against a Winkler titration at the start of sampling season.

DISSOLVED OXYGEN TITRATION KITS

There are several titration kits now available for field use employing the Winkler titration method. LaMotte and Hach are two common kits. Follow manufacturer's directions to obtain accurate results and note the method used to obtain D.O. on the field sheet in the appropriate location. Temperature must be taken at same depth as sample. Take temperature of the grab sample as described in previous section or use a temperature meter with marked cable to take temperature at the same depth as D. 0 sample is taken. Dissolved Oxygen kits should have an accuracy of +/-0.5 ppm.

Record the method used to take D.O. and temperature on field sheet as well as depth of sample. A sampling device designed to take samples at varying depths will have to be used to collect water samples at discrete depths below the surface (e.g. Kemmerer, Van Dorn or equivalent). Use care not to shake the sample in the collection device as this will introduce error into your result. Because of the time and expense involved with using titration kits, larger depth intervals for taking D.O. may be more practical than that outlined previously.

QUALITY CONTROL FOR D.O. WITH TITRATION METHOD

Take duplicate measurements for every 10th sample. Results should not vary more than plus or minus 1 ppm. If results vary greater than 1 ppm, check technique and chemicals used for titration.

PHOSPHORUS AND NITROGEN

BACKGROUND

Phosphorus is one of the major nutrients needed for plant growth. It is generally present in small amounts and limits the plant growth in lakes. As phosphorus increases, the amount of algae also increases. Total phosphorus (TP) samples are taken to characterize the trophic state of a lake. Epilimnetic TP values of 0-7 ppb are found in lakes with low productivity and low algal growth; values between 7-15 ppb indicate that the lake is moderately productive and has moderate algal growth; values above 15 ppb indicate high productivity and high levels of algal growth. Hypolimnetic epilimnion, particularly when D.O. levels are low, can indicate that phosphorus is being released from the sediments and internal recycling of phosphorus may be occurring.

TP samples can be taken at any time of the year, however the most useful information is obtained during the period a lake is most stressed (July-September and February -March). Both grab and core samples are often used to obtain samples for Total Phosphorus (TP) analysis. Most information is obtained from grab samples taken every meter or every other meter throughout the water column. This is the most costly regime, particularly when done every other week during the summer season. In many cases, a TP sample is taken from the epilimnion 1 meter above the sediment in addition to a sample taken 1 meter below the metalimnion. If the hypolimnion is > 10 meters deep an additional sample should be taken in the middle of the hypolimnion to further characterize phosphorus release from the sediments. The latter regime can be done every other week or once a month throughout the summer season. If operating on a very tight budget, a single sample taken in mid-August is recommended.

Nitrogen enters a lake from several sources and in several forms. Figure 11 is a graphical outline showing these sources, as well as the rest of the nitrogen cycle in lakes. Molecular nitrogen (N_2) enters the water from the atmosphere, as do oxygenated forms of nitrogen and organic nitrogen. These oxygenated and organic forms can also enter the lake from tributaries and other influents.

Nitrogen is an essential plant nutrient required by all living plants and animals for building protein. In aquatic ecosystems, nitrogen is present in many different forms. Nitrogen is most abundant in its molecular form (N_2) that makes up 79 percent of the air we breathe. It is a much more abundant nutrient than phosphorus in nature. In its gaseous form (N_2) , nitrogen is useless for most aquatic plant growth. Blue-green algae, the primary algae of algal blooms, are able to use the molecular form of nitrogen (N_2) and biologically convert it to usable forms of nitrogen for aquatic plant growth, ammonia (NH_3) and nitrates (NO_3) . If plants are able to take up ammonia and nitrates through their roots, how do aquatic organisms obtain the nitrogen they need to form proteins? Aquatic organisms eat aquatic plants and convert plant proteins to specific animal proteins or they eat other aquatic organisms that feed upon plants.



Figure 11. Nitrogen exists in several forms in the water, both organic and inorganic. The concentrations of all these forms are important measures of the available nutrients to plants (algae and macrophytes). In the center of Figure 11 are the major inorganic nitrogen forms: nitrate (NO3-), nitrite (NO2-), and ammonium (NH4+)- Various bacteria use these nitrogen molecules and in the process transform the molecules to other nitrogen molecules (through oxidation and reduction reactions). Plants generally use the ammonium and nitrate forms. Ammonium is present in waste products of animals and can be produced from bacterial decomposition. Nitrate is generally mediated by nitrifying bacteria. Some blue-green algae (and bacteria) are able to use nitrogen gas and convert it directly to organic nitrogen (fixation).

As aquatic plants and animals die, bacteria break down large protein molecules into a final product, ammonia. Ammonia is then oxidized (combined with oxygen) by specialized bacteria to form nitrites (NO-2) and nitrates (NO-3). These bacteria get energy for metabolism from oxidation. Excretions of aquatic organisms are very rich in ammonia, although the amount of nitrogen they add to waters is usually small. Duck and geese, however, contribute a heavy load of nitrogen (from excrement) in areas where they are plentiful. Through decomposition of dead plants and animals and excretions of living animals, nitrogen that was previously "locked-up" is now released.

There are even bacteria that can transform nitrates (NO_3) into free molecular nitrogen (N_2) . The molecular form can be lost to the atmosphere. The nitrogen cycle begins again if this molecular nitrogen is converted by blue-green algae into ammonia and nitrates.

Because nitrogen, as ammonia and nitrates, is an important plant nutrient, it also causes eutrophication. Nitrogen rarely limits plant growth (unlike phosphorus), so plants are not as sensitive to increases in ammonia and nitrate levels. As you read in the TSI section, eutrophication causes more plant growth and decay that in turn stimulates a biochemical oxygen demand.

Inadequately treated wastewater from sewage treatment plants; runoff that flushes storm drains containing illegal sanitary sewer connections; and poorly functioning septic systems can also contribute to excess nutrient input. Septic systems are a very common treatment method around rural lakes and in the countryside. Instead of a large, centralized sanitary sewer system which each household is hooked into in urban areas, people with septic systems have individual sewer systems. A septic system is comprised of a main pipe from the house to a box called a septic tank and a number of pipes with holes leading from the septic tank. These pipes are arranged in a grid that usually lies over stone and gravel and is called a "drain field". Wastes from the toilet, kitchen sink, bathtub, and washing machine flow through an underground pipe to a septic tank. In the septic tank, large wastes settle and floating grease is skimmed off. The remaining liquid enters the drain field and is forced out holes in the pipe to trickle through stone, gravel, and soil. In properly functioning septic systems, soil particles remove nutrients like nitrates and phosphates before they reach groundwater. Along some lakes and rivers, septic system drainfields are too near the water table. This reduces the depth of soil and its capacity to remove nitrates and phosphorus.

These nutrients may find their way into the lake or river impoundment via ground water flow. People who neglect septic tank maintenance may allow their tanks to fill with solid materials from previous settling. When the tank is full, household wastes go directly to the drain field instead of settling in the tank. These unsettled wastes can plug the drain field, which prevents filtering of the liquid wastes. In this condition, household sewage may start to pool on the ground and enter water through surface runoff.

Poorly functioning septic systems located close to a well may contaminate groundwater with nitrates. Water containing high nitrate levels, if used for infant milk formula, can cause a condition called methemoglobinemia (methemoglo-bin-emia). This condition prevents an infants blood from carrying oxygen, hence the nickname, "blue-babies". High nitrate levels have also been discovered in groundwater underneath croplands; excessive fertilizer use seems to be the cause, especially in heavily irrigated areas with sandy soils.

Stormwater runoff containing nitrates from lawn and crop fertilizers are responsible for some enrichment of receiving waters. Runoff from feedlots and barnyards can also contain concentrated amounts of ammonia and nitrates.

People have helped create the eutrophication problem that threatens to limit organism diversity, recreational opportunities, and property values—and only we can help reverse eutrophication through thoughtful action.

PHOSPHORUS SAMPLING PROTOCOL

Total Phosphorus (TP)

Samples to be analyzed for TP should be decanted from the field sampling container (raw water) into appropriate sized Nalgene bottles, which have been acid washed and rinsed with distilled water. These bottles are available from any laboratory that is performing the testing. TP samples by themselves need no special handling or filtering after field sample is taken. However, it is common for samples to be analyzed for TP, TN, NH₃, and NO₃ to be sent to the lab in the same bottles (i.e. Wisconsin Laboratory of Hygiene procedures) and as such any filtering or acid preservation must be consistent with or have no effect on all of the individual analytes in the same sample bottle (Table 1 & Fig. 12. App. 2). **NOTE:** Make sure whatever laboratory used is capable of doing low level TP (less than 5 ppb). Phosphorus and nitrogen are

everywhere and very low levels (ppb/ppm) are being measured, therefore, it is important to handle the containers so that the sample is not contaminated (i.e. no fingers inside container, do not let water drip from hands into container, etc.). Refrigeration at 4°C is highly recommended. To prevent contamination, do not remove cover once sample is taken. Samples have a recommend hold time of 24 hours so it is advisable to submit the samples to the lab immediately. It is critically important to label all sample bottles with 1) type of analyte to be tested (i.e. TP, OP, etc.), 2) lake and sampling location, 3) date, 4) depth of sample, 5) and any special sample treatment (i.e. filtering or acid preservation).

Orthophosphate (OP or SRP)

OP samples need special handling after field sample is taken (Table 1 & Fig. 12. App 2). Each sample must be filtered through a .45 micron filter before being sent to the lab. Samples to be analyzed for OP should be decanted from the filtering flask into appropriate size Nalgene bottles, which have been acid washed and rinsed with distilled water. These are available from any laboratory that is performing the testing. **NOTE:** Make sure whatever laboratory used is capable of doing low level 0P (less than 5 ppb). Phosphorus and nitrogen are everywhere and very low levels (ppb/ppm) are being measured, therefore, it is important to handle the containers so that the sample is not contaminated (i.e. no fingers inside container, do not let water drip from hands into container etc.). Refrigeration at 4°C is highly recommended. To prevent contamination, do not remove cover once sample is taken. Samples have a recommend hold time of 48 hours so it is advisable to submit the samples to the lab immediately. Make sure to label sample bottles as noted above.

NITROGEN SAMPLING PROTOCOL

Total Nitrogen (TN)

Samples to be analyzed for TN should be decanted from the field sampling container (raw water) into appropriate sized Nalgene bottles, which have been acid washed and rinsed with distilled water. These bottles are available from any laboratory that is performing the testing. TN samples submitted by themselves need no special handling after sample is taken. However, it is common for samples to be 'analyzed for TP, TN, NH₃, and NO₃ to be sent to the lab in the same bottles (i.e. Wisconsin Laboratory of Hygiene procedures) and as such any filtering or acid preservation must be consistent with or have no effect on all of the individual analytes in the same sample bottle (Table 1 & Fig. 12. App 2). **NOTE:** Make sure whatever laboratory used is capable of doing low level TN (less than 5 ppb). Phosphorus and nitrogen are everywhere and very low levels (ppb/ppm) are being measured, therefore, it is important to handle the containers so that the sample is not contaminated (i.e. no fingers inside container, do not let water drip from hands into container etc.). To prevent contamination, do not remove cover once sample is taken. Samples have a recommend hold time of 24 hours so it is advisable to submit the samples to the lab immediately. Refrigeration at 4°C is highly recommended. It is critically important to label all sample bottles with 1) type of analyte to be tested (i.e. TN, NH₃, etc.), 2) lake and sampling location, 3) date, 4) depth of sample, 5) and any special sample treatment (i.e. filtering or acid preservation).

Ammonia (NH₃₎

Samples to be analyzed for NH₃ should be collected and prepared the same as those for TN (see above). It is common for samples to be analyzed for TP, TN, NH₃, and NO₃ to be sent to the lab in the same bottles

(i.e. Wisconsin Laboratory of Hygiene procedures). Each sample must be filtered through a .45 micron filter before being analyzed but this filtering will be done at the Wisconsin Lab of Hygiene for this project. NH₃ samples must also be preserved with Sulfuric Acid (H_2SO_4) to a pH of less than 2 (Table 1 & Fig. 12. App 2). This preservation is done by adding pre-measured vials if H_2SO_4 to the sample bottle. The addition of H_2SO_4 will not affect the analysis of TN, TP, or NO₃. Make sure to label sample bottles as noted above with particular emphasis on the acid addition.

Nitrate (NO₃)

Samples to be analyzed for NO₃ should be collected and prepared the same as those for NH₃ (see above). It is common for samples to be analyzed for TN, NH₃, and NO₃ to be sent to the lab in the same bottles (i.e. Wisconsin Laboratory of Hygiene procedures). Each sample must be filtered through a .45 micron filter before being analyzed but this filtering will be done at the Wisconsin Lab of Hygiene for this project. Samples must also be preserved with Sulfuric Acid (H₂SO₄) to a pH of less than 2 (Table 1 & Fig. 12. App 2). This preservation is done by adding pre-measured vials if H₂SO₄ to the sample bottle. The addition of H₂SO₄ will not affect the analysis of TN, TP, or NH₃. Make sure to label sample bottles as noted above with particular emphasis on the acid addition.

CHLOROPHYLL (Chl a)

Chl a is a measurement of the green pigment found in all plants including microscopic plants such as algae. It is used as an estimate of algal biomass. Chl a samples are generally taken from epilimnetic samples throughout the summer season every other week or on a monthly basis. When operating on a tight budget, a single mid-August sample is recommended.

SAMPLE CONTAINERS

Sample containers for Chl <u>a</u> can be either glass or plastic. Containers should be large enough to hold 1 liter of sample (about 1 quart). Initially, containers should be soap and water washed and rinsed well to remove all soap residue. After use, the containers need only be rinsed three times to remove sample and allowed to air dry. Rinsing with distilled water is not necessary for these containers. Opaque containers are preferred, however, if using glass or clear plastic containers cover the outside of the container with aluminum foil or keep in the dark such as in a cooler to reduce light penetration during sample handling.

SAMPLE HANDLING

Immediately after collection, Chl <u>a</u> samples should be refrigerated at approximately 4°C and kept in the dark until they are filtered. Filtering should be done as soon as possible, no more than 24 hours after collection. Samples are filtered through GFC plain white 47 mm filters using a Millipore Filtration apparatus and vacuum pump. You may be required to split and filter two equal amounts of the sample so there is a duplicate for quality control and backup purposes. The laboratory procedure used to extract Chl <u>a</u> is the Spectrophotometric Determination found in <u>Standard Methods</u>. The filters should be submitted to the lab as soon as possible so they will be processed within 21 days of sample collection. If unable to deliver filters to the lab on the **day** sample is filtered, wrap filters in foil and freeze the filters in a marked plastic bag until the filters can be delivered. Remember light will harm the Chl <u>a</u> analysis so always keep sample and filters in the dark.

HOW TO COLLECT Chl a SAMPLES

Chl <u>a</u> samples are typically taken from composite epilimnetic water with a VanDorn water sampler. Determine the depth to which the samples will be taken by examining the Temp/D.O. profile. Determine the depth of the true (permanent seasonal) epilimnion. Avoid calling an ephemeral epilimnion, which frequently forms during the summer as a result of a few calm, warm days, the true epilimnion. A good summer rule of thumb is to call all depths warmer than 18 degrees centigrade, part of the true epilimnion, the rational being that it does not take much wind energy or many relatively cool days to mix these layers. Take the sample at 1 meter below the depth of the true epilimnion so as to include algal growth at the epi/metalimnion interface, with one exception: NEVER TAKE A CORE INTO WATER HAVING 2 ppm OF OXYGEN OR LESS FOR CHLOROPHYLL. This could result in high Chl <u>a</u> results from Clorobactor, a bacteria that can be found in waters with 2 ppm D.O. or less.

- 1. Follow instructions for taking field samples with VanDorn sampler. Only one rinse is necessary for Chl <u>a</u>. Be sure to thoroughly drain the sampler on the rinse. When rinsing or collecting water, allow the sampler to remain outside the boat to keep it clean and reduce the risk of sample contamination.
- 2. Lower and raise the sampler SLOWLY.
- 3. Label field sample container with lake name, depth of sample, type (deep vs. grab), date and initials. Pencil is preferred because some inks bleed.
- 4. Decant sampler into field sample container leaving enough air space for agitation prior to filtering.
- 5. Place Chl <u>a</u> sample in a cool dark place immediately to reduce activity of algae in the sample container.

HOW TO FILTER Chl a SAMPLES

- 1. Using forceps (never touch filter with fingers), position a GFC filter on the funnel pedestal (filters are GFC white).
- 2. Rinse the filtering funnel and secure to the pedestal and filtering flask.
- 3. Rinse graduated cylinder with distilled water.
- 4. Shake the sample container vigorously and pour a measured amount of sample into a graduated cylinder. Filter up to a half liter or half of the sample. (NOTE: it is good practice to begin by filtering 250 mls of sample, assessing the amount of time it takes for this to filter, then adding additional sample accordingly, keeping track of the volume added (e.g., if it takes 5 minutes to filter 250 mL, don't add more of the sample; if it takes 1 minute to filter 250 mL, add another 150-250 mL). If a sample is taking more than 2-3 minutes to filter, the funnel should be covered to protect the sample from light sources. When the filtration is complete, remove the funnel.
- 6. Using forceps, fold the filter in half then fold in half again so that filter is about a quarter of original size.

- 7. Place in a square of aluminum foil, fold and label with the lake, date, station, volume of water filtered, and depth of sample. Avoid touching the filter with fingers.
- 8. Store the foil envelopes containing filters in the dark, and as soon as possible, place them in a freezer.

QUALITY ASSURANCE FOR Chi a SAMPLES

Duplicate filters are the only quality control measures currently taking place. It is up to the laboratory to determine how often duplicate filters are run.

CHEMICAL PARAMETERS (pH, Alkalinity, Conductivity, Color)

SAMPLE CONTAINER

Glass or plastic field containers can be used f or collecting pH, Alkalinity, Conductivity and Color samples. Never blow into a container used for pH or alkalinity since the carbon dioxide you exhale could affect the results. Field containers should be washed with soap and water, rinsed well, then rinsed 3X with distilled water. Rinse field container with some sample before filling. Field containers used for pH, Alkalinity, Conductivity, and Color should hold at least 200 mL for each type of sample taken. The same field container can be used for all 4 parameters if it holds approximately 1 liter.

SAMPLING HANDLING

The field container should be filled to the top. Sample should not be shaken. This will introduce error into the pH reading. Filling to the top will reduce shaking of sample during transport. Samples should be kept cold and in the dark to reduce biological activity in the sample. Samples should be analyzed within 24 hours of collection.

A. pH

The pH of a lake influences the plant and animal species present. pH is a measurement of the free hydrogen ion concentration a water sample reflecting how acidic or basic the water is. pH determinations are generally taken on epilimnetic samples to characterize the waters in which algal populations are highest. pH should also be taken near the bottom if possible, since pH will influence the organisms living there. pH levels of 5.0 or lower indicate that a source of acidity is present. Sources of acidity are generally the organic acids released during the decay of humic substances and detritus, but can also be a result of acid deposition. Low pH levels can reduce or eliminate fish populations by reducing reproductive success and by mobilizing toxic forms of aluminum.

pH should be determined in the field if possible. There are several field pH meters available. Follow manufacturers directions for obtaining correct reading with pH meter. It is necessary to make sure that the probe used is for solutions with low ionic strength. Most pH probes are for high ionic strength waste water and may not be as accurate for lake water.

If measuring in the lab, the first sample removed from the chemistry container should be for the pH determination.

Record pH and method used to determine pH on field form. If using a meter, round to 2 decimal places. If using a calorimetric method, then record to one place after decimal.

QUALITY CONTROL FOR pH

Calibrate pH on two buffers bracketing the suspected pH (usually 4.0 and 7.0). Prepare buffers according to manufacturer's instructions. After calibration, check that the probe is functioning properly by first measuring the pH of aerated, de-ionized water. The meter should return a value of 5.65. As a second check, measure the pH of a weak acid with a known pH. To obtain a solution with a pH of 4.7, using volumetric glassware, dilute 1 ml of 0.02 N sulfuric acid (H_2SO_4) to I liter.

Take care to thoroughly rinse the probe with distilled water prior to and following each step. When doing pH in the field, measure temperature as well as pH, unless the meter is temperature compensated. Depending on the. meter used, results should not vary plus or minus 0.2 units. If the results vary more than 0.2, check buffers, check probe and check meter for the source of error.

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B. SPECIFIC CONDUCTANCE (CONDUCTIVITY)

Conductivity is a measure of the ability of water to carry an electrical current and is directly related to the dissolved ions (charged particles) present in water. Specific conductance is conductivity corrected to 25^oC. Generally values are low in Midwest lakes (20-60 microsiemens per centimeter (uS/cm). Specific conductance is generally determined on epilimnetic samples. Specific conductance should also be taken near the bottom if possible, since specific conductance will influence the organisms living there. Results are often used by fisheries biologists to help predict productivity of fish populations in a lake.

Specific conductance can be measured with your portable meter or a meter in a lab. The portable meter has a built in thermister which automatically corrects for temperature. Other meters lacks this feature, so samples analyzed by other means should be brought to 25 degrees centigrade in a water bath prior to measuring conductivity or the temperature must be taken and recorded at the time of analysis so that a correction can be made. Probes also may have a multiplier (i.e. 10x or 100x) that must be used to determine the final value. It is important to know if the results have been corrected for temperature as specific conductance will increase by 2% for every degree increase in temperature.

Record the specific conductance on the field form. The result recorded must be corrected to a temperature of 25 degrees centigrade.

QUALITY CONTROL FOR CONDUCTIVITY

Take duplicate samples after every 10th sample. Results should not vary more than 2 uS/cm. Check meter on a known standard before using. Check that de-ionized water reads less than I uS/cm. Rinse probe thoroughly between use. Follow manufacturers advice for storage, cleaning and use of probe.

C. ALKALINITY

Alkalinity is a measure of the amount of calcium carbonate equivalent in the water, which reflects the ability of the water to buffer pH changes. Generally, alkalinity is determined on epilimnetic core samples to characterize the waters in which algal populations are highest. Alkalinity should also be determined on a sample taken near the bottom if possible, since alkalinity will influence the organisms living there.

QUALITY CONTROL FOR ALKALINITY

Take duplicate samples after every 10th sample. Results should not vary more than 1 mg/L. If results do vary, check that steps were followed exactly. Repeat test. If results still exceed allowable limits do not record results. Try to find source of error. **NOTE:** It is highly recommended that 2 results within +/-1 mg/L of each other even if 3 to 4 titration's are required.

D. APPARENT COLOR

The amount of "color" in a lake refers to the concentration of natural dissolved organic acids such as tannins and lignins, which give the water a tea color. Lakes that are considered colored (>25 SPU) can have reduced transparency readings and increased phosphorus values. Generally, epilimnetic core samples are used for color determinations because these are the waters which have the most potential for interfering with Secchi transparency readings. Apparent color should also be determined from samples taken near the bottom if possible, since it may provide insight into chemistry of those waters and may influence the organisms living there

MISCELLANEOUS PARAMETERS

A. PHYTOPLANKTON

Phytoplankton measurements are valuable indicators of water quality, because the species and abundance present have been correlated to distinct water quality conditions, with some species thriving in eutrophic systems and others showing sensitivity to various pollutants. The presence of blue-green algae, their relative abundance and bloom frequency, is a primary qualitative indicator of degrading trophic status.

Whole water samples (250 ml) are collected and immediately preserved with Lugol's iodine. The color of the water after addition of Lugol's should be the color of fine cognac. Sample date, location, and depth are noted. Bottles need only be clean and after preservation samples can be stored at room temperature.

B. NEARSHORE BACTERIA SAMPLING

Bacteria are trapped in the pore space between soil particles under septic systems. This entrapment or filtration is an important mechanism for removal of enteric bacteria from effluent. A clogging mat occurs at the interface between the drainage field and natural soils. This mat is formed in part because of bacterial activity and serves in turn to help trap enteric bacteria before entering the soil. Other important factors influencing the attenuation of bacteria include bacterial numbers in the effluent, soil texture, soil saturation, loading rates, temperature and bacterial type. Unsaturated flow beneath drainfields is important in ensuring slow travel, long residence time for bacteria in the unsaturated zone, good aeration, increased opportunity for contact between effluent and soil particles, opportunity for absorption of bacteria to soil particles and eventual die-off of bacteria.

As stated above, soil conditions, effluent loading, treatment system age and proximity to the water resources is of concern in the removal of enteric bacteria. The presence of these bacteria in groundwater is not only an indication of leaking treatment systems but is also a human health concern to lake property owners through potential contamination of drinking water wells.

Non-pathogenic *Fecal coliform* and *Fecal streptococcus* bacteria reside in the intestines of all warmblooded animals, including humans. Appearance of these organisms in lake samples may indicate contamination from sewage, industrial wastes or land runoff. *Fecal coliform/Fecal streptococcus* ratios may give an indication of the source of pollution. A ratio greater than 4.1 may indicate pollution from human waste while ratios of less than 0.7 imply pollution from non-human sources.

Bacterial sampling consists of dip samples collected by inverting the bottle (neck-down) in the water and inverting to fill, 6 in. below the surface of the water. All bacterial samples should be collected away from the edge of the boat using a gloved hand technique. Samples should be packaged on ice and delivered to the Wisconsin Department of Hygiene's laboratory for analysis. Samples should be sent by UPS overnight service.

C. WELL WATER SAMPLING

Early in the history of public health, man recognized that water was a carrier of many of our worst diseases. The quality of water for communities and individual households is a source of concern for all of us, since diseases can be contracted from contaminated water sources and then spread to others in the community.

Most county Health Departments offer laboratory analysis and evaluation of private drinking water sources. "Drinkability" of water generally means the absence of harmful bacteria or chemicals and can usually be determined by laboratory tests. However, a. safe water supply is not only defined by the absence of these contaminants but the reasonable assurance that the supply will not become contaminated in the future. Therefore, a well must also be properly constructed, located and operated.

The Water Sample

A sample from your water system is collected and analyzed in the laboratory. Even if no indicators of contamination are found, it is a good practice to submit another sample at a later date. It is recommended that individual wells be tested a minimum of twice annually until a sequence of tests are negative, after which time the frequency may be reduced to once annually. At least one of these samples should be

collected during the summer months when the ground is not frozen. Any noticeable changes in water quality such as turbidity, color, taste, odor, etc., indicate the need for immediate investigation and testing. If you want to have your water tested, contact the County Health Department.

Sources of Contamination

If your water is contaminated, it is usually due to a problem with the water source or the construction of the well itself. Well water is drawn from a water bearing formation, which may become polluted by surface water, sewage or by chemical wastes. Your well, pump and distribution system may allow pollutants to enter the water supply.

Lab Analysis

The basic test for drinkability consists of two tests - Total Coliform bacteria and Nitrate Nitrogen. The results of your water test(s) will be compared to existing standards to determine if your water is acceptable for drinking.

Basic Laboratory Tests for Drinking Quality

- 1. Total Coliform -- coliform bacteria are found in the soil and in the intestines of man and other animals. Therefore, if this bacteria is found, it indicates the possibility of surface water or sewage contamination. Test results should show a total coliform count of less than regulatory limits (<1/100 ml.) of water to be acceptable.
- 2. Nitrate Nitrogen -- nitrates are the end products of the decomposition of plant and animal matter. It may also occur naturally in the ground. Contamination of a sewage or fertilizer origin should be suspected if results show an excess of 1 mg./liter. Sewage and fertilizers may be associated with other chemicals not tested *for* by this department. Concentrations of 10 mg./liter or more may be dangerous to infants. If the level is above 1.0 mg./liter, the water should be tested again within the next month and periodically thereafter to determine if the levels are increasing.

Disinfection and Retesting

If any coliform bacteria are found, (or non-coliform bacteria in excess of 200/100 ml.) the water supply should be inspected to detect any defects or deviations from appropriate construction, location and operational standards. Upon correction of these deviations the well should then be disinfected.

Common Causes of Contamination

The presence of contaminated water in a well is commonly associated with the following:

- 1. The well is located too close to sources of contamination (septic system disposal fields, buried sewer lines, barnyards, chemical spills, etc.)
- 2. The well casing terminates in a pit. Well pits often fill with surface water after heavy rains or during the spring runoff. The water in the pit then flows into the well often without the homeowner realizing that it has occurred.

- 3. The well casing has cracks, holes or open joints (this is particularly true with dug wells constructed of concrete or clay tile sections). Openings in a well casing may allow the entry of surface water, insects, rodents and other sources of contamination.
- 4. The well does not have an adequate cover. This occurs primarily with dug wells. Every well should have a watertight overlapping cover that prevents the entrance of water, insects, rodents and other sources of contamination.

NOTE: Large diameter wells can be a hazard, especially to children. Design the cover to prevent accidents.

- 5. The ground adjacent to the well casing is low, allowing surface water to accumulate around the well. In some instances this water follows the casing to the groundwater supply. Clay or silty soil should be mounded around the casing to divert surface water away from the well.
- 6. Concrete slabs surrounding well casings often have voids under the slab which permits the accumulation of water adjacent to the well casing, resulting in problems similar to item 5 above.
- 7. The water source itself is contaminated or is otherwise unsuitable for drinking.
- 8. Drilled wells constructed in areas that have bedrock near the ground surface may be contaminated by surface water due to inadequate soil cover, which filters and purifies the water.
- 9. The well and distribution system have not been thoroughly disinfected following installation, repair or modification.
- 10. Improper design of household plumbing may allow back-siphonage of contaminated water into the well or distribution system.

D. INORGANIC METALS (Calcium, Iron, Magnesium, Manganese, Sodium, Etc.)

Discrete samples for metals analysis should be collected once a year from the normal lake sampling site, for the surface grab and the deep sample. Samples should be collected with a PVC Van Dorn water sampler that has been checked for trace levels of metals. For determination of "total" metals a raw water sample is placed in pre-labeled Teflon or plastic bottle containing the sampling date, lake name, site number and depth (provided by the State Laboratory of Hygiene). Samples are preserved according to instructions provided by the State Laboratory of Hygiene to below pH 2 using a solution of nitric acid. For "dissolved" metals, a 125 ml sample is filtered using a 0.45 um nucleopore filter (Table 1 & Fig. 12. App 2). Samples should be placed into coolers and kept dark and chilled during transport to the laboratory by overnight express UPS.