Standard Operating Procedure for Phytoplankton Analysis

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1.0 Scope and Application

This method is utilized to identify, enumerate and measure phytoplankton taxa in samples collected from the Great Lakes. Algal taxa are identified to the lowest taxonomic rank possible. A listing of all organisms identified and their respective density and morphometric measurement for biovolume calculation is reported.

2.0 Summary of Method

The method consists of two parts - analysis of phytoplankton (excluding most diatoms) and analysis of diatom. For operational reasons, the first part of the analysis is also called "soft algae" analysis. The "soft algae" are defined as those that are either naked or have a cellulosic cell wall and cannot withstand acid digestion treatment. In contrast, diatoms have relatively "hard" silicious valves and the valves can tolerate harsh acid treatment. Initially a preliminary scan is made of a settled 10 mL sample in order to determine the volume to be used for each of the two analyses. For the soft algae analysis, organisms are enumerated in a settling chamber using an inverted microscope at 500x magnification. For diatom analyses, the samples are pretreated with strong oxidants and the cleaned samples are mounted on glass slides and enumerated using a compound microscope at 1250x magnification.

3.0 Sample Collection and Preservation

3.1 See United States Environmental Protection Agency Great Lakes Analytical Contract Operation Procedure for phytoplankton sample collection and preservation.

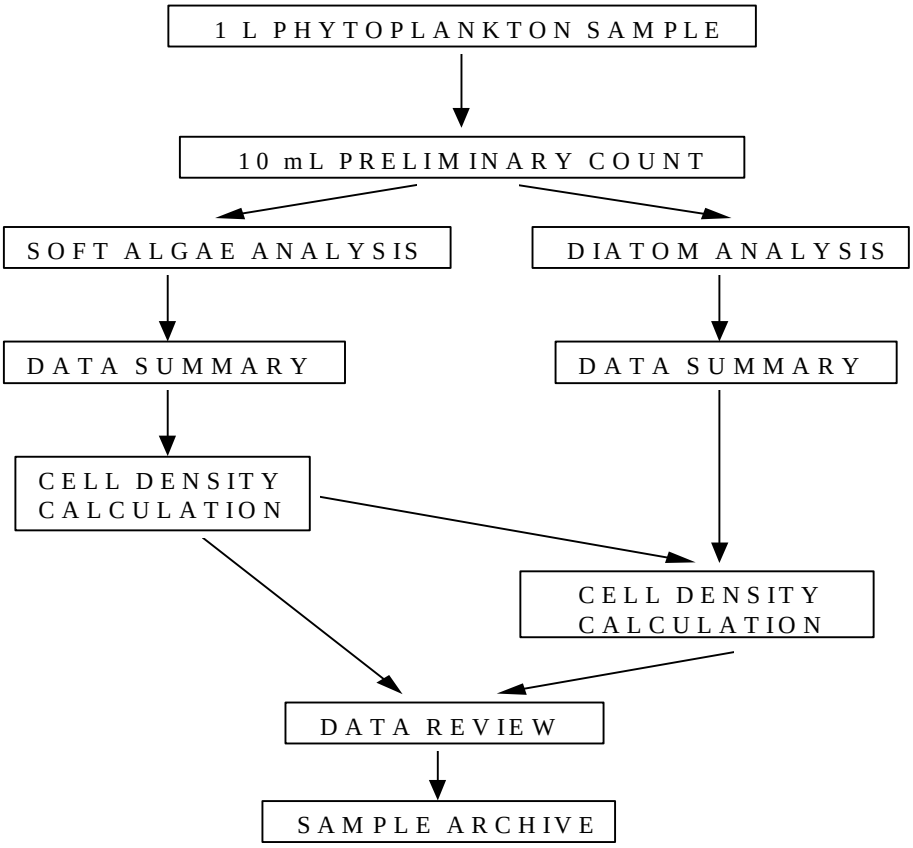
3.2 After the preserved phytoplankton samples arrive at the laboratory from the survey, an additional 10 mL of Formalin is added to each sample to enhance the storage life of the sample.

3.3 All sample containers and diatom slides must be properly labeled as follows:

a. Sample containers: Lake: Station, CRL and LAB Number; Sampling Date; Sample Type (Integrated, B-1, B-2..etc.).

b. Diatom slides: Lake; Station, CRL and LAB Number.

Note: CRL numbers are assigned by GLNPO to all samples-collected in the field, LAB numbers are assigned by the contractor in the laboratory, for internal use only, to facilitate the sample log-in and identification procedure. Each sample has its own CRL Number that corresponds to a specific LAB Number (see United States Environmental Protection Agency Phytoplankton and Zooplankton Sample Log-in Standard Operating Procedure).



4.0 Determination of Sample Volume Required for Analyses

4.1 10 mL Preliminary Investigation

The 10 mL preliminary investigation is usually performed by the soft algae analysts in order to determine the appropriate volume of sample required for both soft algae and diatom analyses.

4.1.1 Apparatus

4.1.1.1 lnverted microscope with an objective system for magnification up to 150x (Leitz Diavert or another equal quality inverted microscope).

4.1.1.2 Tubular plankton chamber or combined plate chamber 10 cc.

4.1.1.3 Cover plate for plankton chamber, 33 mm dia., 2 mm thick.

4.1.1.4 Base plate for plankton chamber, 27.5 mm dia., 0.2 mm thick.

4.1.1.5 10 mL automacropipette.

4.1.2 Procedure

This procedure is done by settling 10mL of each sample and counting the total number of organisms and number of diatom cells within a 10 mm^2 area. No identifications are done at this time but any irregularities such as excessive sediment in the sample are noted. All information from the 10 mL preliminary count is recorded in a pre-printed data form (Appendix 1). This includes unusual observations such as poor sample preservation, high bacterial or fungal populations, occurrence of special or rare phytoplankton taxa ... etc.

Note: The definition of an organism for 10 mL preliminary counts is as follows:

A colony, a filament, or a single cell. The units of a colony or a filament are not counted as organisms at this time but the whole aggregate is counted as one organism.

Note: 10 mm^2 = One transect from edge of chamber to edge of chamber at 250x.

4.2 Determination of Sample Volume Settled

4.2.1 There is no exact limit set for determining the volume needed, each sample is examined for the number of organisms present, amount of debris in the sample and its distribution pattern. Large amount of debris often require that smaller then optimal volumes be settled.

4.2.2 Most samples are settled at 10 or 25 mL, with 25 mL being the usual volume. Only when samples are difficult or impossible to count are 5 mL or 2.5 mL samples used. The 50 mL samples are used when very low number of organisms are found in the samples.

4.2.3 The volume needed for setting (soft algae analysis) and for digestion (diatom analysis) is determined from the number of all organisms counted during the 10 mL preliminary investigation. However, the minimum volume for digestion is recommended to be 500 mL. For example:

10 mL preliminary counts

1) 101 organisms total
2) 103 diatom cells (Note: 1 cell has two frustules or valves)

Count needed (minimum)

1) 250 organisms total
2) 500 diatom frustules (250 cells)

Final volumes

1) 25 mL sample for sedimentation
2) 500 mL sample for digestion

4.2.4 The final volume may be slightly over-estimated to ensure that the minimum counts required are met. The preliminary count also helps to ensure that there is enough sample for both final investigations.

5.0 Sample Analyses

Samples are analyzed by data set, and a QC count is chosen for 10% of the samples in each set. The QC is chosen by the Team Leader who takes into account the 10 mL preliminary data and the diatom counts, if available.

5.1 Soft Algae Sample Analysis

Organisms are identified to the lowest taxonomic rank possible. Characteristics such as size, shape, color and the presence of flagella are used in the identification process. Any obscure or unidentifiable organisms are checked by the Team Leader or one other analyst. Drawings are made of the organism, complete with all sample identifiers (i.e. LAB and CRL numbers, Station number, Survey number, and analyst's initials). The drawing is then added to the permanent card file in the lab, and may also be sent out to other specialists for identification or verification. The card file is reviewed frequently and any additional information is added as received.

5.1.1 Apparatus

5.1.1.1 Inverted microscope with an objective system for magnification up to 600x (Leitz Diavert or another equal quality inverted microscope)

5.1.1.2 Tubular plankton chamber or combined plate chamber 10 cc.

5.1.1.3 Cover plate for plankton chamber, 33 mm dia., 2 mm thick

5.1.1.4 Base plate for plankton chamber, 27.5 mm dia., 0.2 mm thick

5.1.1.5 10 mL automacropipette

5.1.1.6 Syringe 20 mL with cannula, 14 gauge 4 inch

5.1.1.7 Long-neck disposable pipettes

5.1.1.8 Rubber bulbs for pipettes

5.1.2 Analytical procedures

5.1.2.1 Sample Sedimentation

The phytoplankton sample is mixed by gently inverting the sample bottle for 60 seconds. The predetermined sample volume (see Section 3.0) is loaded into a sedimentation chamber of appropriate volume. Samples should be added to the

chamber with a syringe (less than 10 mL) or macropipettor (10 mL or more). The sample bottle should be inverted at least once between each addition. This is done because larger organisms settle quickly and may remain in the bottle if the sample is simply poured. The chamber is topped with a round glass top plate.

5.1.2.2 Sample Settling

Algae are allowed to settle onto the base of the settling chamber. Since oil immersion may be used in the course of identification, the coverglass at the bottom of the chamber should not be thicker than 0.2 - 0.3 mm in thickness (or No. 1 coverglass). The time recommended for complete sedimentation varies with the height of the chamber, i.e. 8 cm/day to 4 cm/day depending on accuracy required in enumeration (Furet & Benson-Evans, 1982).

Approximate settling times necessary are as follow:

100 mL	----------	100 hours
50 mL	----------	50 hours
25 mL	----------	25 hours
10 mL	----------	10 hours
5 mL	----------	5 hours
2 mL	----------	2 hours

5.1.2.3 Sedimented Sample Analysis

Only "live" forms (chloroplast containing organisms) are counted and identified at 500x. Higher magnification may be used for identification when necessary.

5.1.2.3.1 The chamber of settled material is scanned and the dominant (four or five most common organisms) as well as subdominant taxa are determined. This is to give the analyst an idea of the sample composition as well as to insure that the sample is evenly settled.

5.1.2.3.2 Enumeration and identification are done by scanning parallel strips of 10 mm per strip (each strip has a width of 0.2 mm which gives an area of 2 mm^2). A minimum of three strips (30 mm or 6 mm^2) is required, including no less than 250 "live" organisms. If 250 organisms are not observed within the three strips, identification and enumeration are continued in strips until at least 250 are counted. The area counted is recorded as it is needed for cells per mL calculation.

5.1.2.3.3 The number of "live" cells are identified and enumerated to the lowest taxonomic rank possible. All "emptied" lorica from Chrysophyta are also identified and enumerated.

5.1.2.3.4 At least 10 specimens of each taxa are measured for cell volume calculations. When fewer than 10 specimens are present those present are measured as they occur. The measurements required are those which are necessary for the volume calculation of a solid which best approximates the shape of any particular organism. For most organisms the measurements are taken from outside wall to outside wall.

Those forms which are loricate (e.g., selected members of Chlorophyta, Euglenophyta and Chrysophyta) must have the active portion, i.e. protoplast, measured. Empty lorica are also counted, but not measured. Filamentous and colonial forms require measurements of the individual components.

5.1.2.3.5 Diatom cells are counted while making the strip counts at 500x. At this magnification the diatoms are enumerated and identified only as live pennates, empty pennates, live centrics, and empty centrics. Actual identification of diatoms and cell volume measurements are done under oil immersion (1250x) by another method (see Section 5.2). The only diatoms which must be counted at 500x are: Asterioneila formosa, Fragilaria capucina, Fragilaria crotonensis, Tabellaria flocculosa, Rhizosolenia eriensis and species of Rhizosolenia longiseta.

5.1.3 Archiving

Soft algae samples are to be archived one data set at a time.

5.1.3.1 Gently mix the remainder of the phytoplankton sample by repeatedly inverting the bottle for about one minute. Carefully empty the sample into a 500 mL graduated cylinder and cover the cylinder with a plastic Petri plate. Record the volume of sample settled on a pre-printed phytoplankton archive form (Appendix 6). A larger and/or smaller graduated cylinder may be used depending on the volume remaining in phytoplankton sample bottle.

5.1.3.2 Rinse the sample bottle three times with a small amount of RO/DI or distilled water (about 5 mL). Empty the rinse water into the graduated cylinder.

5.1.3.3 Settle the sample for a minimum of seven days, but not more than 14 days. Do not disturb the cylinder.

5.1.3.4 At the end of the settling period, carefully siphon off the top of the water column without disturbing the settled materials. Generally, about 18-22 mL of the sample should be remaining in the cylinder.

5.1.3.5 Decant the remaining sample from the graduated cylinder into a pre-labeled 25 mL glass liquid scintillation vial. Rinse the cylinder two times with about 2 mL of RO/DI or distilled water and empty the rinse water into the vial. This is the archived sample.

5.1.3.6 Add about 0.5 mL of Formalin solution to the archived sample before putting the cap on the vial.

5.1.3.7 Store the archived sample in a pre-labeled tray/box.

5.1.3.8 Record the archived sample information (CRL number, lab number, station number, original volume and concentrated volume) into the computer using DBASE III + data management program.

5.2 Diatom Sample Analysis

Diatom identifications and enumerations are performed on prepared slides. Because the cellular contents of diatoms obscure the wall markings on which the taxonomy is based, the organic matters inside the cell must be removed (oxidized) prior to identification.

5.2.1 Apparatus

5.2.1.1 Research quality compound microscope with an objective system of magnification up to 1400x (Letiz Dialux or another equal or better quality compound microscope).

5.2.1.2 Beakers - 300 and 600 mL

5.2.1.3 Hotplate

5.2.1.4 Centrifuge

5.2.1.5 Centrifuge tubes, graduated 15 mL

5.2.1.6 Cover slips, round, #1 thickness, 22 mm diam.

5.2.1.7 Precleaned microscope slides, 25 X 75 mm.

5.2.1.8 Long-neck disposable pasteur pipettes

5.2.1.9 Rubber bulbs for pipettes

5.2.1.10 Slide Warmer

5.2.2 Reagents

5.2.2.1 HNO_3 Nitric Acid (concentrated)

5.2.2.2 H_2O_2 Hydrogen peroxide (30% solution)

Hydrogen peroxide must be kept in an air-tight container, store in dim light or in the dark, and in a refrigerator.

5.2.2.3 $K_2Cr_2O_7$ Potassium dichromate

5.2.2.4 Hyrax™ mounting media

5.2.2.5 Toluene or Xylene

5.2.2.6 "Leitz" immersion oil

5.2.3 Cleaning Of Diatoms Valves and Slides Preparation

This section describes a method for cleaning diatom valves and preparing permanent diatom slides.

5.2.3.1 Cleaning of Diatoms

The first three steps of the diatom cleaning procedure must be carried out under the hood.

5.2.3.1.1 A specified volume (see Section 4.0) of uniformly mixed sample is poured into a 600 mL beaker. The recommended minimum volume is 500 mL. Mix the sample by gently inverting the sample bottle for a minimum of one minute.

5.2.3.1.2 Add 20 mL of concentrated HNO_3 to digest organic matter in the sample. Place beaker on a hot plate and concentrate sample to approximately 20 mL by heat evaporation. Allow sample to cool and transfer to a 300 mL beaker. Rinse the side of the 600 mL beaker several times with DI/RO water and transfer the rinse water to the digested sample.

5.2.3.1.3 Adjust the volume of the digested sample to 150 mL with DI/RO water. Further oxidize the sample with 25 mL of 30% H_2O_2. Accelerate the process by adding a few grains of $K_2Cr_2O_7$. Place beaker on a hot plate and concentrate sample to approximately 10 mL by heat evaporation. Allow the sample to cool and transfer to a 15 mL graduated centrifuge tube.

5.2.3.1.4 Rinse the side of the beaker several times with DI/RO water and transfer the rinse water to the centrifuge tube. Fill the tube with DI/RO water and centrifuge at low speed (1500 rpm) for 30 minutes.

5.2.3.1.5 Draw off all but 0.5 mL of supernatant in the centrifuge tube using a vacuum system. Take care not to disturb the pellet at the bottom of the tube. Add approximately 10 mL of DI/RO water to the tube and gently shake the sample using a vortex mixer. Recentrifuge the sample for 30 minutes at low speed (1500 rpm). Repeat Step 5.2.3.1.5 10 times.

5.2.3.1.6 Upon final centrifugation draw off all but 0.5 mL of supernatant. Bring volume up to approximately 5 mL with DI water. This is the "cleaned" sample to be used to prepare diatom slide for analysis.

5.2.3.2 Diatom Slide Preparation

Where possible, two duplicate slides should be made from each sample. The second slide will be sent to a repository at a later date.

5.2.3.2.1 Place a clean coverslip (thickness: No. 1; size 22 mm, circular) on a slide warmer (150-200°F).

5.2.3.2.2 Gently mix the sample and pipette about 0.25 mL aliquot of the sample on a coverslip and let dry. Examine the dried coverslip under the microscope. If the diatom density is not sufficient for counting, dry more sample on to the coverslip.

5.2.3.2.3 Add a small drop of Hyrax mounting medium to the center of a clean prelabeled slide (75 X 25 mm). If the Hyrax mounting medium is too viscous, add a few drops of toluene and/or xylene to dilute the medium.

5.2.3.2.4 Mount the coverslip, diatom side down, on the slide and place on hotplate.

5.2.3.2.5 Allow solvent to evaporate until bubbles are no longer formed under the coverslip. Remove from the hotplate.

5.2.3.2.6 Press coverslip gently with pencil eraser to extrude excess Hyrax immediately after removing from heat as the medium sets up very quickly.

5.2.3.2.7 Allow the slide to cool and remove excess Hyrax before examining. It will scrape away easily with a razor blade if all of the solvent is removed: if it is sticky, return to the hotplate to remove any remaining solvent.

5.2.3.2.8 Clean and label (CRL number, LAB number, Station number) the slide.

5.2.4 Diatom Enumeration and Identification

Diatoms are identified and enumerated to lowest taxonomic rank possible at 1250x.

5.2.4.1 A minimum of 500 frustules is counted (2 frustules = 1 diatom cell) per sample (slide).

5.2.4.2 At least 10 specimens of each taxa are measured (wall to wall) for cell volume calculations. When fewer than 10 specimens are present, those present are measured as they occur (Appendices 4 & 5).

5.2.5 Archiving

5.2.5.1 After the diatom slides are made, transfer the remainder of “cleaned" sample to a pre-labeled 9 mL glass vial.

5.2.5.2 Store the diatom archived sample in a box for future reference.

6.0 Calculations

6.1 Report the results of the sample sedimentation procedure as cells per mL which is calculated as follows:

$$cells/mL = \frac{C \times TA}{L \times W \times V \times S}$$

Where: C = *cell count*
L = *length of strip (mm)*
W = *width of strip (mm)*
V = *volume of chamber (mL)*
S = *number of strips counted*
TA = *total area of chamber bottom (mm^2)*

Note: Calculation factor listed at the bottom of Appendix 3 is equal to:

$$\frac{TA}{L \times W \times V \times S}$$

6.2 Reasonable approximations of geometric shape and mean dimensions will be reported so that cell volume estimates can be determined.

6.3 The data from the diatom slides is reported as percent composition of the 1250x count. This percent is applied back to the diatom counts at 500x to determine a cells/mL count for each species.

6.3.1 Calculate the total live diatom cells/mL as per formula in Step 6.1.

6.3.2 Calculate the percent composition of the pennate and centric diatom taxa on the prepared slide by dividing the number observe by the total pennate and total centric diatom values enumerated respectively.

6.3.3 Calculate the cells/mL for each diatom taxon by multiplying the total live pennate and centric diatom cells/mL (from the soft algae analysis) by the percent pennate or centric diatom counts respectively (from the diatom analysis).

7.0 Quality Control and Method Precision

7.1 Ten percent of all samples collected are analyzed in duplicate. At least one duplicate count is done per data set if the data set contains less than 10 samples. This includes identification, and tabulation of data. Data shall be calculated for the groups below:

Cyanophyta	Other minor divisions
Chlorophyta	Indeterminable forms
Chrysophyta	Pennate Diatoms
Cryptophyta	Centric Diatoms
	Total phytoplankton

The relative percent difference (RPC)) between duplicate determinations shall be compared to the guidelines listed below:

Cyanophyta (Picoplankton + Cyanophyta)	56%	
Chlorophyta	82	*
Chrysophyta	87	*
Cryptophyta	52	
Others	22	
Unidentified	75	
Pennales (Live + Empty)	80	***
Centrales (Live + Empty)	72	****
Total	48	

* Cells must number >140 before RPO guideline can be applied
** Cells must number >198 before RPD guideline can be applied
*** Cells must number >98 before RPO guideline can be applied
**** Cells must number >274 before RPD guideline can be applied

$$RPD = \frac{(larger\ value) - (smaller\ value)}{Average\ value} \times 100$$

7.2 Determinations which exceed the control guidelines listed above may require re-analysis unless:

7.2.1 The RPO value is the result of low density (especially true for the other minor divisions category).

7.2.2 The RPO value is the result of chance occurrence of colonial forms which are enumerated as individuals thus skewing the population estimate.

7.2.3 Other reasonable explanations can be provided to explain the differences between counts.

7.3 Previously calculated RPD values are used to determine the consistency of the identifications between analysts at the division level, as they only compare total cell numbers and not actual species identifications.

7.3.1 If the calculated values fall outside RPD Guidelines and no explanation can be found, the sample may be reanalyzed by either or both analysts or a third analyst, where necessary.

7.3.2 If the sample data are accepted by the analysts, they are then submitted to the Team Leader for his or her approval.

7.4 Photographic and Line-drawing Record

Photographic records of diatom and other phytoplankton taxa should be taken. Resulting positive prints should be enlarged to a specific diameter (i.e. 1000x) and attached to 5 x 8 index cards or 8½ x 11" sheets. The card must contain the following information:

a) Taxon name with dimensions and magnification
b) Photograph with negative reference number (if any)
c) Sampling date and location
d) Location of specimen on slide (diatoms only)
e) Slide identification number (diatoms only)
f) Comments
g) Name of analyst

Example format:

Taxon:_____ Photograph(s)/Line Drawing(s)

Dimensions: ___X___µm

Comments: _____

_____

Slide ID: _____ Location: _____

Sampling date: _____ Analyst: _____

This continuously updated file serves as the quality control reference document for diatom and other phytoplankton taxa. The file also serves as reference standard for the questionable and unidentifiable forms.

8.0 Safety and Waste Disposal

Proper PPE should be worn in the laboratory while handling and preparing samples for analysis,

especially during the digestion process. Follow all laboratory waste disposal guidelines regarding the disposal of acid waste. Do not discard samples containing acid into the sink. All waste should be placed in a designated, and labeled, waste drum.

9.0 References

9.1 Standard Operating Procedure For The Analysis Of Phytoplankton - U.S. E.P.A. - GLNPO 1987. Prepared by the Bionetics Corporation.

9.2 Furet, J.E. and K. Benson-Evans. 1982. An evaluation of the time required to obtain sedimentation of fixed algal particles prior to enumeration. Br. Phycol. J. 17: 253-258.

9.3 U.S. E.P.A. Great Lakes Program Office. 1987. Analytical Contract Operation Procedure for Phytoplankton Sample Collection and Preservation.

9.4 Utermohl, H. 1958. Zur vervoilkommnung der quantitativen phytoplankton-methodik. Mitt. lnt. Ver. Limnol. 9. 38 pp.

9.5 Phytoplankton Sampling And Preservation Standard Operating Procedure - U.S. E.P.A. 1994. Prepared by the Enviroscience Corporation.

Appendix 1. Great Lakes - Phytoplankton Samples

Preliminary Investigation
 # Organisms in 10 mL - Min. Area = 15 mm^2

Sample Number	Station # & Depth	# Organisms		Total Vol. Needed	Comments
		>10 µm	<10 µm		

Appendix 2. Phytoplankton Bench Sheet

Sample Number _____ Lake _____
Lab Number _____ Analyzed by _____
Station & Depth _____ Date Analyzed _____
Date Collected _____ Method _____
Data Set Number _____ Volume Analyzed _____

Cell Tally

	PICOPLANKTON - spheres	Sweep 1 = Sweep 2 = Sweep 3 =
	PICOPLANKTON - rods	
	UNIDENTIFIED OVOID - flagellates ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	UNIDENTIFIED SPHERICAL - flagellates ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	RHODOMONAS MINUTA VAR. MANNOPLANCTICA ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	COCCOID - OVOID ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	COCCOID - SPHERES ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	ANACYSTIS MONTANA f MINOR ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	
	HAPTOPHYTES SPP ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )/	

Appendix 3. Phytoplankton Analysis

Lake _____ Sample Number _____
Analyzed by _____ Lab Number _____
Data analyzed _____ Station & Depth _____
Method _____ Date Collected _____

TOTALS	@ 500x		
Picoplankton			_____ cells/mL
Cyanophyta (Blue-greens)			_____ cells/mL
coccoids	_____		
filaments	_____		
Chlorophyta (Greens)			_____ cells/mL
coccoids	_____		
filaments	_____		
flagellates	_____		
Desmids	_____		
Chysophyta (Golden Browns)			_____ cells/mL
coccoids	_____		
flagellates	_____		
Haptophytes	_____		
colorless flagellates	_____		
Cryptophyta			_____ cells/mL
Pyrrhopnyta (dinoflagellates)			_____ cells/mL
Euglenophyta (Euglenoids)			_____ cells/mL
Xanthophyta (Yellow greens)			_____ cells/mL
Chloromonadocnyta (Chloromonads)			_____ cells/mL
Unidentified flagellates and coccoids			_____ cells/mL
Eacillariophyta (Diatoms-Live Cells)			_____ cells/mL
Rhizosoinia spp.			_____ cells/mL
live pennates	_____		_____ live cells/mL
empty pennates	_____		
live centrics	_____		_____ empty cells/mL
empty centrics	_____		
diatom valves (@ 1250x)	_____	=	_____ frustules

Dominant species

_____
_____
_____
_____
_____

Area scanned_____mm^2
Volume settled_____mL Total . . . . . _____cells/mL
Calculation factor _____

Calculation factors for phytoplankton samples

	Volume of Sample Settled				
Strips	5 mL	10 mL	25 mL	50 mL	100 mL
1	49.0875	24.5438	9.8175	4.9088	2.4544
2	24.5438	12.2719	4.9088	2.4544	1.2272
3	16.3625	8.1813	3.2725	1.6363	0.8181
4	12.2719	6.1359	2.4544	1.2272	0.6136
5	9.8175	4.9088	1.9635	0.9818	0.4909
6	8.1813	4.0906	1.6363	0.8181	0.4091
7	7.0125	3.5063	1.4025	0.7013	0.3506

Appendix 4. Phytoplankton Analysis

Lake ______________________
Analyzed by ______________________
Data analyzed ______________________
Method ______________________

Sample Number ______________________
Lab Number ______________________
Station Number______________________
Date Collected ______________________

TAXON	*Cell Tally*	*Cells per mL*	*Cell Shape*	*Average Cell Dimensions*				*Cells Measured*
				Length	*Width*	*Depth*	*Diameter*	

Appendix 5. Quality Control Data Sheet
Relative Percent Difference
Phytoplankton

CRUISE _____ CRL # _____ LAB # _____

STATION # _____

D.S. #

DIVISION	COUNT 1	COUNT 2	RPD	LIMITS
CYANOPHYTA				56
CHLOROPHYTA				82*
CHRYSOPHYTA				87**
CRYPTOPHYTA				52
others				<23
unidentified				75
PENNALES				80***
CENTRALES				72****
total				48

* Cells must number >140
** Cells must number >198
*** Cells must number >98
**** Cells must number >274

Appendix 6. Phytoplankton Archive Data

	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L		8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L
	8 - 0 _ _ _ 8 G _ _ _ I _ _ conc. from mL orig. Vol. 1 L				

Appendix 7. Phytoplankton Archive Labels

89-0081
89GA20I12
LM 17
conc. from 41 mL
orig. vol. 1 L

89-0082
89GA20I32
LM 11
conc. from 522 mL
orig. vol. 1 L

89-0083
89GA20I52
LM 18
conc. from 477 mL
orig. vol. 1 L

89-0084
89GA20I72
LM 19
conc. from 435 mL
orig. vol. 1 L

89-0085
89GA20I92
LM 23
conc. from 445 mL
orig. vol. 1 L

89-0086
89GA21I12
LO 27
conc. from 505 mL
orig. vol. 1 L

89-0088
89GA21I12
LM 32
conc. from 487 mL
orig. vol. 1 L

00-0000
00XX00X00
XX 00
conc. from 000 mL
orig. vol. 1 L

00-0000
00XX00X00
XX 00
conc. from 000 mL
orig. vol. 1 L

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00XX00X00
XX 00
conc. from 000 mL
orig. vol. 1 L

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00XX00X00
XX 00
conc. from 000 mL
orig. vol. 1 L

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XX 00
conc. from 000 mL
orig. vol. 1 L

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orig. vol. 1 L

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orig. vol. 1 L

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orig. vol. 1 L

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orig. vol. 1 L

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conc. from 000 mL
orig. vol. 1 L