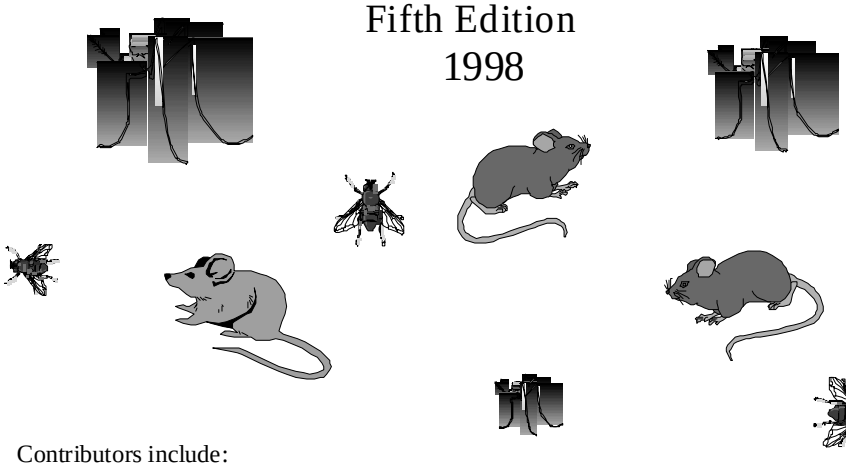
METHODS OF THE

CASCADING TROPHIC INTERACTIONS PROJECT

Fifth Edition

1998



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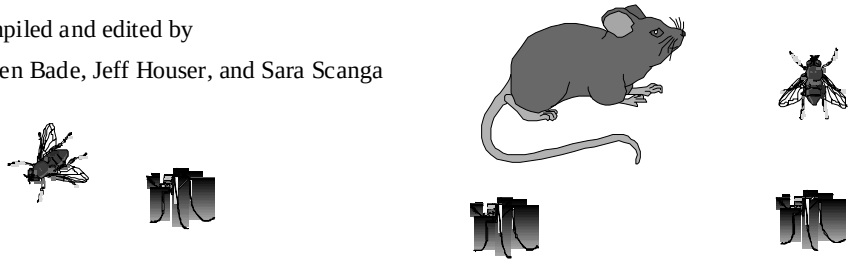

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disclaimer: These methods have been used successfully in the lakes studied by the Trophic Cascade Project. However, we cannot guarantee that they will work in lakes with different water chemistries from those we have studied. While we have made reasonable efforts to error-check these documents, we cannot guarantee that they are error free. We disclaim responsibility for any errors in these documents.

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PREFACE TO THE FIFTH EDITION (1998)

The Cascading Trophic Interactions Project is an experimental study of the interaction of nutrient flux and food web controls in regulating biological and chemical processes. In this stage of the project, four basins (East Long, West Long, Peter and Paul Lakes) are included in a two by two factorial design testing the interaction of nutrient levels and community structure. Two adjunct projects are investigating microbial and benthic processes in the experimental lakes. This research builds on a whole lake manipulation experiments started in 1985 involving Peter, Paul and Tuesday Lakes. These lakes are all located within the University of Notre Dame Environmental Research Center in Gogebic County, Michigan. Each summer, the lakes in the current study are sampled weekly, with different parameters and different frequencies according to the procedures described in this manual.

Many changes in the fifth edition have been made to accommodate the increasing teamwork between IES and the Limno Team. Therefore revisions can be seen in the field sampling protocol, as well as the routine lab procedures. Major change has taken place in the GC procedure which now includes gas standards and other new techniques documented by Nick Voichick and Dave Thomas. Sara Scanga was responsible for the revisions and additions to the now more complete microbial processes section. Darren Bade created the new section detailing fertilizer methods used in previous years. A large portion of this manual was written in previous editions and remains unchanged or has undergone modest revisions. Several methods have become outdated but remain in this edition for quick reference and include a note as to when they were last in use. Darren Bade, Sara Scanga and Jeff Houser all aided in proofing and checking to make this one of the best editions yet. Thanks to all who have contributed to this manual in the past, present and future. THE TRUTH IS OUT THERE.

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ROUTINE FIELD SAMPLING (as performed in 1997)

Paul, Peter, West Long, East Long and Tuesday Lakes are sampled weekly at permanent sampling stations on transect lines over the deepest portion of each lake. One lake is sampled per day.

1997 sampling schedule:

Monday - Paul Lake
Tuesday - Peter Lake
Wednesday - West Long Lake
Thursday - East Long Lake
Friday - Tuesday Lake

A. Water Collection Crew

Equipment:

Van Dorn Sampler
6 Light Depth Bottles - 2L Nalgene bottles
1 Hypo. Bottle - 2L Nalgene bottle
1 Meta. Bottle - 2L Nalgene bottle
1 PML Bottle - 4L Nalgene bottle
1 BOD Bottle - 300mL (for pH)
1 Rite In The Rain notebook
Pencil
Oars
Life Preservers
Seat Cushions

1. Rinse all bottles with water from their respective depths 3 times before collection.

2. Using 4L Van Dorn, collect fixed depth water (from Pooled Mixed Layer (PML), Metalimnion, and Hypolimnion). Depths to sample are as follows:

Bottle (total volume to collect)	**Lake, Depth(meters)**			
	Paul	Peter	West	East
PML (4L total, equal vol. from each depth)	0,1,2 m	0,1,2 m	0,1,2 m	0,0.5,1 meters
Meta (2L total)	4 m	4 m	4 m	2 meters (if oxygen <1 at 2m collect at 1.5m)
Hypo (2L total)	9 m	12 m	12 m	12 m

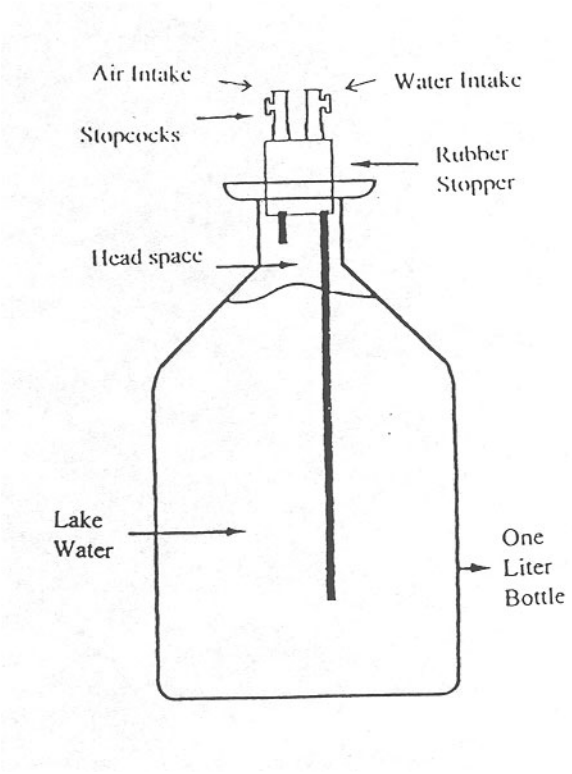
3. Collect surface water pH sample using BOD bottle by dipping bottle over side of boat.

4. Collect light depth determined water, (2L per depth with sampling depths determined by Isotope Crew) using 4L Van Dorn.

B. Isotope Crew

Equipment:

O2/Temp meter (YSI)
Light Meter Probe (LiCor)
Light Meter Box (containing data logger, extra data sheets and pencil)
Leucine Insulators - 6
Leucine sample cups - 4
Leucine 60mL BOD bottles - 2
Water pump
Life Preservers, oars, seat cushions
Pencil
Clipboard with instructions and data sheet
Zooplankton net, 2 zooplankton cups, 4oz. live count jar, 4 oz. jar for preserved sample, squirt bottle filled with tap water
Secchi Disc
PPR box (every other week); 60 ml BOD bottles and stoppers - 18+1 extra, C14, DCMU, Pipettor, Gloves, Waste bag
Sediment trap supplies (every other week, alternating with PPR) : 2 - 2L Nalgene bottles, pickle bucket lid, squirt bottle
2 L polycarbonate bottle
Double-valve stem stopper
3 60 ml syringes with stopcocks
4 20 ml syringes with stopcocks



1. Rain Gauge: Record reading(cm) on data sheet

2. Sonde Deployment: Move sondes (and anemometer) to appropriate locations (note that on Wednesday and Friday, separate crew may perform this work)

Sonde (and anemometer) Schedule:

Day	Task
Monday	Program sondes first thing in the morning. Deploy sondes in Paul and Peter. Deploy anemometer in Peter Lake
Wednesday	Move sondes to East and West Long Lakes
Friday	Pick up sondes from East and West Long Lakes and anemometer from Peter Lake. Download information on computer.

3. Staff Gauge: Record reading on data sheet

4. Light Profile (using LiCor submersible light meter, deck light meter, and data logger): Lower sensor from sunny side of the boat, taking readings at the surface and at 0.25 m intervals to 1.0 m, then at 0.5 m intervals to 1% surface irradiance. Calculate the 6 depths of 100, 50, 25, 10, 5, and 1% surface irradiance.

5. PPR (every other week, alternating with Sediment Trap retrieval): Pump water from each light depths into 3, 60 ml BOD bottles Follow **14C Primary Productivity** instructions in this manual.

6. Sediment Traps (every other week, alternating with PPR weeks): see **Sediment Trap Sampling** in this manual>

7. Temperature-Dissolved Oxygen profile (using YSI digital meter and probe): Calibrate DO meter following the instructions on the back of the unit. Take DO and temp. readings at 0.5 m intervals from 0-7 m, then at 1 m intervals to: 12.0 m from East Long, West Long, Peter, and Tuesday Lakes; and 8.0 m for Paul Lake. Record DO and temperature to the nearest 0.1 unit.

8. Zooplankton Hauls: take 2 vertical hauls for zooplankton with zooplankton net. Lower the net to the appropriate depth and retrieve at a constant rate of 3 seconds per meter. Pool both tows into zooplankton jar to be preserved at the lab. Put a 3rd haul into the jar labeled “live counts.” Thoroughly rinse the zooplankton cup into the appropriate sample jar after each tow using the squirt bottle filled with tap water. See the following chart for depths of zooplankton hauls for each particular lake.

Zooplankton Haul depths:

Paul Lake - 8 m
Peter Lake - 12 m
West Long Lake - 12m
East Long Lake - 12m
Tuesday Lake - 12m

9. Take a Secchi disk reading from the shady side of the boat. Lower the disk until it disappears, note the depth, raise the disk until it reappears, note that depth, and average the two readings.

10. Using the pump, collect water for Leucine Incubations from 6 depths listed in table below. Rinse and fill sample containers for oxic depths (plastic cups) and place them inside of thermos container. Fill thermos container with water from the same depth as the sample. Rinse and fill 60 mL BOD bottles (for anoxic depths). Fill bottles completely with pump on low speed. **Be certain** that no air bubbles are present in the anoxic bottles. Insert glass caps. To prevent leucine contamination from your flesh, do not touch the bottom end of the cap. Place the 2 BOD bottles in an insulating container and fill with water from an anoxic depth.

Leucine Incubation Depths:

Lake	**Depths (m) (*last two depths use anoxic container*)**
Paul	1,2,3,4,*5,9*
Peter	1,2,4,5,*7,9*
West	1,2,3,4,*6,9*
East	0.5,1,1.5,2,*4,9*

11. pCO2: Before starting, check all syringes for leaks by closing their stopcocks and pressing the plunger down. If no leaks are heard and pressure is maintained in the syringes, continue. If there is leakage, replace syringe. Fill 2, 60 mL syringes with air from windward direction. Close stopcock on one and place in cooler. Rinse and fill 2 L polycarbonate bottle with surface water and cap with double-valve stem stopper, leaving both valves open. See figure in the beginning of this section. Insert one air-filled 60 mL syringe into the air-intake stopcock (short tube inside bottle) and one empty 60 mL syringe into the water-intake stopcock (long tube inside bottle). Slowly inject 60 mL air while removing 60 mL water without creating a vacuum or increased pressure. Close both stopcocks and remove syringes. Shake bottle 100 times to equilibrate head space. Insert water filled syringe into water-intake valve. Attach empty 20 ml syringe into the air-intake valve. Open stopcocks on both syringes and stopper, and remove 20 ml air while inserting the same volume of water. Close the stopcock on the full 20 mL air filled syringe and detach. While holding syringe with the stopcock end down, quickly open and close valve to remove water and equilize pressure. Place in cooler. Remove another 20ml air sample with second syringe and equalize pressure in the same manner as the sample before.

Empty the polycarbonate bottle and repeat the entire operation once more.

(labproc.doc)

ROUTINE LIMNOLOGICAL LABRATORY PROCEDURES

These procedures are best preformed immediately after the field procedures in the morning.

1. Rinse zooplankton net (srubbing with hands while rinsing) and cup. Preserve zooplankton by adding Lugol's solution until sample turns a light brandy color. See recipe for Lugol's solution in **Zooplankton Sampling and Counting.**

2. Make ~ 1.5 L of pooled mixed layer water (PML) by combining equal amounts of water from the top three depths.

3. Process phytoplankton samples: Fill 2, 250 mL brown (opaque) plastic bottles with PML water. Add 2-3 mL glutaraldehyde (under the hood, wearing gloves) and refrigerate.

4. Determine DIC for the 6 depths (in duplicate), using the gas chromatograph (see **Gas Chromatograph Method for Measurement of DIC**).

5. Filter water samples from each of the 6 depths onto a GF/F filter (these are the total chlorophyll samples).

   A. Filters have a grid side and a smooth side. Place filter smooth side up.

   B. Shake sample bottle well before filtering. Filter enough volume so the at the filter is discolored.

   C. Record filter volume

6. Rinse filter towers and filters with distilled water, place filters in labeled film canisters and place in freezer. Labels should include lake, day, and depth ID (1-6).

7. Repeat steps 3 and 4 but first pre-filter sample through a 35 µm mesh. These are the "edible" chlorophyll samples.

   A. Filter 50 mL more for 35 µm filtered sample than for non-prefiltered sample.

   B. Film canister should be labeled "F" (as well as lake, day, and depth) to indicate a pre-filtered sample.

8. Nutrient sample preparation: See also **Nutrient Analysis.**

   A. Total P and total N: Shake lake water collection bottle well. Rinse 3X and pour about 100 mL into each of two acid-washed 125 mL bottles. Label

bottle with lake, day , depth code (1-7), and TN or TP. Freeze. Once per week, add Milli-Q to a bottle as a "blank" sample.

B. Dissolved nutrient analysis:

i) Remove filtering flask from acid bath and rinse with Milli-Q.

ii) Use a new GF/F filter for each sample depth. Filter some sample, swirl to rinse flask, discard this rinse. Repeat for 3 rinses, then filter approximately 200 mL.

iii) First rinse each of two acid-washed 125 mL bottles 3 times with filtered sample and then transfer about 75-100 mL of filtered sample to each bottle.

iv) Once a week filter a Milli-Q "blank" after depth 6 to check method. Filter the hypolimnion sample last (depth 7).

v) Label bottles with lake, day, depth code (1-7), and NO3+PO4 or NH34.

vi) Freeze samples.

vii) Return filtering flask to 2N HCl acid bath.

9. Count *Conochilus* colonies as well as Daphnia in the live zooplankton sample under a counting scope.

10. If doing AER, APA, gran alkalinity, or pH, see instructions in appropriate sections of this manual.

IN THE AFTERNOON

1. Chlorophyll, see **Chlorophyll *a* Analysis**.

2. PPR or AER, see instructions in appropriate sections of this manual.

NUTRIENT ANALYSES

General Procedures For All Analyses:

EQUIPMENT AND SUPPLIES:

Lachat AE autoanalyzer
100-1000 µL Eppendorf pipettor and non-sterile tips
1-5 mL Oxford pipettor and non-sterile tips
5-10 mL Oxford pipettor and non-sterile tips
13 X 100 mm disposable glass tubes
Balance (0.1 mg sensitivity)
Balance (0.1 g sensitivity)
Nalgene bottles for reagents (500 mL, 1000 mL), dark and light bottles, acid washed
Nalgene bottles for standards (125 mL), acid washed
Nalgene bottles for samples (125 mL), acid washed
Scintillation vials, acid washed (2N HCl)
Milli-Q water
Helium
Acid Wash - 2N HCl

SAMPLE AND EQUIPMENT PREPARATION:

A. ACID WASHING:

NOTE Take efforts to avoid contamination at all stages: washing bottles and apparatus; preparing standards and reagents; transferring samples; etc. This means acid wash everything, preferably within a day of performing the analysis. Also, do not use spatulas in reagent bottles if a reagent can be shaken out. Never touch any sample, reagent or the business side of a vessel or tool with fingers.

1. Soak bottles, glassware, etc. overnight in 2N HCL. Always rinse items with tap water before putting in acid bath.

2. Remove items from acid bath, rinse 3 times with Milli-Q (no tap rinse), and let air dry.

3. Smaller items to be stored for more than a few days should be kept in zip lock bags. Otherwise, invert glassware or cover with parafilm or foil to avoid contaminat

B. BOTTLE PREPARATION:

1. Acid wash 125 mL Nalgene bottles before collection. These sample bottles may be reused but must be acid washed between samples.

2. Each bottle should be labeled with lake code (L=Paul, R=Peter, E=East Long, W=West Long, C=Central Long, T=Tuesday), Julian day, depth code (1 for surface, 7 for hypolimnion), and analysis to be performed (e.g. L183 6 TP). The types of analysis are as follows: a) $NO_{2,3}$ & OPO_4 - dissolved nitrates and orthophosphates; b) $NH_{3,4}$ - dissolved ammonium; c) TP - total nitrogen; d) TN - total phophorus.

3. Soak the filtering flask for dissolved nutrients in 2N HCl overnight. Reserve the acid solution between uses.

C. SAMPLE PREPARATION (Day of Collection):

1. **Dissolved Nutrient Analyses:** nitrate plus nitrite, ammonia , and ortho-phosphate or soluble reactive phosphorus .

   a. Rinse the flask 3-4 times with Milli-Q (no tap).

   b. Filter small amount of sample using a GF/F filter, and rinse collection flask 3 times. Filter more sample (approx. 75 mL) into flask and use small amount to rinse acid washed sample bottle 3 times. Transfer remainder of sample (approx. 50 mL) into rinsed bottle. Dispose of filter.

   c. To avoid cross contamination, filter depths 1 through 7 consecutively. Filter Milli-Q between depths 6 and 7 (for a blank).

   d. Freeze samples.

2. **Total Nutrient Analysis**: TP and TN

   a. Shake lake water collection bottle well. Rinse acid washed sample bottle 3 times with sample. Pour about 100 mL of sample into bottle. 1 time per week: use Milli-Q to make 1 blank bottle. Freeze samples.

D. PREPARATION OF STANDARDS:

1. Stock Standard Preparation Procedure:

a. Weigh quantity of dry standard (see Detailed Methods) and record the amount

b. Dissolve dry standard in 1L volumetric flask in about 800 mL Milli-Q. Dilute to the mark with Milli-Q, and invert many times.

c. Determine actual concentration of N or P according to the formula: (g compound weighed/(g/mole compound))*(gram molecular weight element/mole element) = g/L element in solution.

2. Working Standard Preparation: (see Detailed Methods)

Following is a recipe chart for making working standards from stock standards:

Target Conc.	Stock Vol.	Tot. Vol. (Stock+Milli-Q)	Stock Conc.
5000 µg/L	5 mL	100 mL	100000 µg/L
1000 µg/L	1 mL	100 mL	100000 µg/L
750 µg/L	15 mL	100 mL	5000 µg/L
500 µg/L	10 mL	100 mL	5000 µg/L
400 µg/L	8 mL	100 mL	5000 µg/L
300 µg/L	6 mL	100 mL	5000 µg/L
200 µg/L	4 mL	100 mL	5000 µg/L
100 µg/L	2 mL	100 mL	5000 µg/L
75 µg/L	7.5 mL	100 mL	1000 µg/L
50 µg/L	5.0 mL	100 mL	1000 µg/L
25 µg/L	2.5 mL	100 mL	1000 µg/L
15 µg/L	1.5 mL	100 mL	1000 µg/L
10 µg/L	1.0 mL	100 mL	1000 µg/L
8 µg/L	0.8 mL	100 mL	1000 µg/L
5 µg/L	0.5 mL	100 mL	1000 µg/L
2 µg/L	0.2 mL	100 mL	1000 µg/L

E. LACHAT HARDWARE AND SOFTWARE:

Refer to the Lachat manuals or the following Detailed Methods section for a more specific or thorough description.

1. **Hardware**

There are five main parts to the Lachat.

a. Sampler: The sampler automatically sips sample from the sample tubes. There are three racks for sample tubes. The fourth rack from the left is labeled "empty tubes", and samples should not be placed in a rack in this position. To the right of the sample racks is a rack for the standards, used to generate the standard curve. This rack has positions A (front left) to N (back right) and holds scintillation vials.

b. Pump: a peristaltic pump that carries samples and reagents to manifolds

c. AE: The central and largest piece of apparatus, it has two channels for manifolds, a computer (with a seldom used keyboard resting on top of the AE), and a monitor.

d. IBM computer: This is a DOS computer equipped with software for running the AE.

e. Printer: The printer is used to print reports and other data output, or can act as a chart recorder to capture output seen on the AE monitor. A switch box (between the AE and the IBM) controls whether the printer is to be used as a printer or a chart recorder.

2. **Software:**

a. Method

"Method" is the Lachat term for the software file defined by the user under "Method Definition", and is used to drive the AE for a particular analysis. Any number of methods can be defined for any analysis. For example, there may be a method defined to run OPO4 on channel 1 and two others to run OPO4 on channel 2. Each method is actually a subdirectory on the computer with data files created using that method in the same subdirectory. **NOTE** If you call up a method and make any changes to it, be sure to save changes and then download the method, even if a method with the same name was already downloaded. The method with the most recent changes must be downloaded for the recent changes to be incorporated into your next run.

ABBREVIATED NUTRIENT ANALYSIS INSTRUCTIONS

The following is a quick reference for the order of procedures used when running any nutrient analysis.

1. Thaw samples.
2. Acid wash (make sure that have adequate amount of Milli-Q for entire analysis).
3. Turn on autoanalyzer (heater should warm up for 45 min.), and inspect initial screen (look for upper case OK's).
4. Install manifold.
5. Make standards.
6. Digest samples and standards (if analyzing for total nutrients).
7. Make reagents.
8. Enter standard concentrations and sample ID's into computer.
9. Degas reagents (except for NO_3 analysis).
10. Run reagents through autoanalyzer to establish baseline.
11. Download Method.
12. Pour standards and calibrate autoanalyzer (run standards).
13. Pour and run samples (assuming that calibration has been approved).
14. Check sample data and rerun samples if necessary.
15. Save the data onto a floppy disc.
16. Shut down manifold (follow method specific guidelines).
17. Shut down / clean up.
    a. release manifold cassettes from pump.
    b. put manifold lines in plastic bag.
    c. empty waste.
    d turn off helium.
    f. turn off autoanalyzer.
    g. soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.
    h. discard samples or store samples in freezer.

TROUBLE SHOOTING

This is a list of the most commonly encountered problems when using the Lachat autoanalyzer, along with possible causes and solutions.

A. No Peaks

1. Is the pump on correct speed?

2. Are reagent lines in reagents? Make sure lines are taped to the bottles so the line intake tip is well below the reagent fluid level.

3. Be sure that the standards are in descending order and that you have correctly specified their placement in the standard rack in the method definition (see Appendix IV).

4. Is the sample line running to Port 6 and the waste water line running out of Port 5 **on the valve of the correct channel**?

5. Are you using the channel defined in the method you have downloaded?

6. Is the correct filter in (correct filters are listed on manifold diagrams posted on wall next to machine)?

7. Check for leaks in sample and reagent lines.

8. If running NO_3, is cadmium column in line?

9. Sometimes there is a clog in one of the lines (possibly from incomplete digestion in TP or TN analysis), causing reagents and/or carrier to flow backwards. Use dye (green food color dye kept on shelf above and to the left of the AE) to make sure all reagents and carrier are flowing freely and flowing forward. To avoid cross contamination of reagents, first flush Milli-Q through all lines, then run dye through individual lines to determine where clog exists. It is also possible to determine leaks by introducing an air bubble in each line and watching it progress.

10. Check results (see Appendix IV). It is possible that the curves were present but out of view.

B. Poor Calibration Curve

1. Check all items in Step A.

2. Calibration problems are almost always due to contamination problems that can be avoided by the following precautions:

a. Make standards fresh weekly. Acid wash standard bottles between uses.
b. Acid wash scintillation vials that hold standards. For dissolved nutrient analyses, rinse vial well with standard before pouring standard into vial. This is not possible with digested standards (for TP and TN) because there is not enough processed standard for rinsing.
c. Use only freshly acid washed degassing wands and use only the appropriately labeled wand in each reagent. Be sure that any part of the wand that will be in the reagent is prevented from contacting any surface (especially fingers).
d. If analyzing for TP or TN, be sure that the digestion tubes have been freshly (within a few days) acid washed.
e. Cover reagents with parafilm when in use. A phosphate and a nitrate reagent contain ammonia and a nitrate reagent contains phosphoric acid which could cause cross-contamination.
f. Be sure that standard values were typed correctly, in descending order, into method definition. Also check the boundary conditions to be sure that there is at least 1 standard between each boundary (at least 3 standards per segment). Otherwise, each of the standards will be sipped twice by the sampler. If standard concentrations are changed, be sure to save the changes and download the method, even if a method with the same name was already downloaded.

C. Defining a New Method: Check Standards

Check standards can causes complications in the operation of the Lachat, thus using check standards is not recommended. One alternative is to run standards with the samples, in the sample tray (after calibration has been approved). Currently defined methods do not contain check standards. Use the following steps in the Lachat software to avoid using check standards when defining a new method:

1. Under Methods > Method definition > Channels > Standards > Check Standards > Triggers: change # samples between check standards to a large number; change # trays between check standards to a large number; change # hours between check standards to a large number.
2. Under Methods > Method Definition > Channels > Standards > Check Standards > Actions: specify "yes", which results in user deciding if a re-calibration should be done.

DETAILED METHODS

Dissolved Nutrients

NOTE Usually, all the dissolved nutrient analyses are performed on the same day. In order to avoid airborne cross contamination from reagents of other analyses, perform the dissolved nutrient analyses in the following order: **Ammonia → Phosphate → Nitrate**.

AMMONIA

Method No. (10-107-06-2-B)
Range: 0.5-100µg/L
Method Name: AMMONIA (ammonia ch 1)

****NOTE**Check Method No. in Lachat Manual for most current method in use. If Method No. is different than that listed above, follow instructions in Lachat Manual and determine the current method name.**

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Chemicals:

Sodium hydroxide (NaOH)
Sodium potassium tartrate (d,l-$NaKC_4H_4O_6*H_2O$)
Sodium phosphate dibasic heptahydrate ($Na_2HPO_4*7H_2O$)
Sodium salicylate [salicylic acid sodium salt] $C_6H_4(OH)(COO)Na$]
Sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO*2H_2O$
Regular Chlorox Bleach (5.25% sodium hypochlorite)
Ammonium chloride (NH_4Cl) (standard)

Analysis Instructions:

1. **Thaw samples**.
2. **Acid Wash** items necessary for analysis.**NOTE** Both fans should be turned on throughout entire procedure except when using the balance (0.1 mg sensitivity).
3. **Turn on Autoanalyzer** (heater should warm up for 45 min.), and inspect initial screen (look for large cased OK's)
4. **Install Manifold**: on Channel 1. Refer to the diagrams on the wall to the left of the sampler to make sure that the manifold is correctly connected to the AE unit. In particular, pay attention to the diagram describing the connection and disconnection of the carrier line to the valve.**NOTE** Make sure the sample line is connected to the valve on the correct channel.

5. **Make Standards**:

a. **Stock Standard** (see Prepartation of Standards)
Dry Standard Target = 3.819 g ammonium chloride (NH_4Cl), which has been dried at 110°C for 2 hours, to make 1000 mg N/L stock solution.

b. **Working Stock Standards and Working Standards**
Working stock standards and working standards are made within a few days of the analysis (preferably the same day).

1. Prepare working stock standards (usually 5000 and 1000 µg/L) from the stock standard.
2. Prepare working standards from the working stock standards. Working standard concentrations should be in the range of expected sample concentrations (determined from past data). For example: 2, 5, 8, 10, 15, 25, 50, 75, 100 µg/L
   a. Pick a target concentration (e.g. 1000 µg/L) and determine the mass of Stock Standard (or Working Stock Standard) needed to achieve the target (e.g. 1 g) (see chart in Preparation of Standards).
   b. Tare a 125 mL standards bottle and add the estimated mass of stock solution
   c. Record the actual mass of stock used (e.g. 1.0387 g), and add enough Milli-Q to make approximately 100 g. Record the total mass (e.g. 99.5026 g).
   d. Calculate the final concentration: (actual mass / total mass)*****stock concentration

   (e.g. (1.0387g / 99.5026g) ***** 100327µg/L = 1047.31µg/L).

Example:

Target Conc.	Est. Stock Wt.	Act. Stock Wt.	Total Wt.	Stock Conc.	Final Conc.
1000 µg/L	1 g	1.0387 g	99.5026 g	100327 µg/L	1047.31 µg/L

6. **Make Reagents**

A. **BUFFER**: Combine 28 g **sodium hydroxide** (NaOH), 50.0 g **sodium potassium tartrate** (d,l-$NaKC_4H_4O_6*4H_2O$), 134 g **sodium phosphate dibasic heptahydrate** ($Na_2HPO_4*7H_2O$) and 885 g **Milli-Q** (for a final mass 1097g). Shake or stir until dissolved. .

B. **SALICYLATE-NITROPRUSSIDE COLOR REAGENT**: Combine 144 g **sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], 0.29 g **sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO*2H_2O$] and 856 g **Milli-Q** (for a final mass 1000.29g). **Store in a dark Nalgene bottle**. Shake or stir until dissolved. **Prepare at least weekly**. For 40 samples you will need about 500g, see table below:

Salicylate	Nitroprusside	Milli-Q	Total mass
144 g	0.29 g	856 g	1000.29 g
72 g	0.145 g	428 g	500.145 g
36 g	0.0725 g	214 g	250.0725 g

C. **HYPOCHLORITE REAGENT**: Combine 64 g regular **Chlorox Bleach** (5.25%sodium hypochlorite) and 936 g **Milli-Q**. Mix well.

D. **CARRIER: Milli-Q**

7. **Enter Standard Concentrations and Sample ID's into Computer:** Enter standard concentrations in descending order and establish boundaries of the segments with at least one standard between two boundaries. Then enter sample ID's in the Sample Menu (see Appendix IV).
8. **Degas All Reagents** (including carrier) with helium for 2 minutes using appropriately labeled wands.
9. **Run Reagents Through Autoanalyzer** Snap cassettes onto pump with lines in place and arrows on cassettes pointing towards manifold. Rinse all manifold lines with Milli-Q before putting them in reagents. Cover reagents with parafilm and tape lines to bottles. Turn pump on "normal" speed (**NOTE** Dial next to pump should be set at 35). Check to be sure that reagents are not leaking and flowing freely by following air bubbles through individual lines. Be certain that there is enough of each reagent for the entire run. If additional reagents are added (with the exception of Milli-Q), a new calibration should be performed (see 12.).
10. **Download Method** (see Appendix IV). Baseline should be steady. An unsteady baseline indicates air in the reagents; degas them again.
11. **Pour Standards** into scintillation vials and place them in the standard rack in descending order.
12. **Calibrate Autoanalyzer**; i.e. run standards (see Appendix IV).
    a. If calibration is approved, start running samples.
    b. If calibration is not approved, look at calibration statistics and decide to either:
        1. Approve calibration (see Appendix IV).
        2. Redo calibration curve (perhaps with new reagents and/or new standards). See Trouble Shooting.

13. **Pour and Run Samples** (assuming that calibration has been approved). Pour samples into 13*100 mm tubes and insert them in the sampler rack. Place the rack in the position specified in the method. Submit the samples (see Appendix IV).
14. **Check Sample Data** and rerun samples if necessary
15. **Save the Data on Disc**. To copy only report data, see Appendix IV. To copy all raw data including the calibration curve, use the following DOS command:

    C:> COPY C:\LACHAT\METHODS\"method name"'\"file date"*.* A:

    For example:

    C:> COPY C:\LACHAT\METHODS\NEWTP2\940406*.* A:

    NOTE To reenter the Lachat menu from DOS, type "menu", or "mm" when in the subdirectory C:\LACHAT.
16. **Shut Down Manifold**
    a. At the end of the run, place all lines, **except buffer line,** in Milli-Q and flush system for one to two minutes.
    b. Place buffer line in Milli-Q (do not use this Milli-Q for anything other than flushing) and pump for another two to three minutes
    c. If a clog (or potential clog) is suspected, run HCl (1 or 2 M) through all lines for 2-3 minutes. Then flush again with Milli-Q for 2-3 minutes.
    d. Pump all lines dry.
17. **Shut Down/Clean-Up**
    a. Release manifold cassettes from pump.
    b. Put manifold lines in plastic bag
    c. Empty waste.
    d Turn off helium.
    e. Turn off fans.
    f. Turn off autoanalyzer.
    g. Soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.

h. discard samples or store samples in freezer.

PHOSPHATE

Method No. (10-115-01-1-B)
Range: 1.0-200µg/L
Method Name: NEWOPO4 (pilot OPO4 ch 2)
Method in use since 7/93

****NOTE**Check Method No. in Lachat Manual for most current method in use. If Method No. is different than that listed above, follow instructions in Lachat Manual and determine the current method name.**

Chemicals:

H_2SO_4
Ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}*4H_2O$]
Antimony potassium tartrate [$K(SbO)C_4H_4O_6*½H_2O$]
Ascorbic acid
Dodecyl sodium sulfate [$CH_3(CH_2)_{11}OSO_3Na$]
Sodium hydroxide (NaOH)
Tetrasodium ethylenediamine tetraacetic acid (Na_4EDTA)
Anhydrous potassium dihydrogen phosphate (KH_2PO_4) (standard)

Analysis Instructions:

1. **Thaw samples**.
2. **Acid Wash** items necessary for analysis.**NOTE** Both fans should be turned on throughout entire procedure except when using the balance (0.1 mg sensitivity).
3. **Turn on Autoanalyzer** (heater should warm up for 45 min.), and inspect initial screen (look for large cased OK's)
4. **Install Manifold** on Channel 2. Refer to the diagrams on the wall to the left of the sampler to make sure that the manifold is correctly connected to the AE unit. In particular, pay attention to the diagram describing the connection and disconnection of the carrier line to the valve.**NOTE** Make sure the sample line is connected to the valve on the correct channel.
5. **Make Standards**:
    a. **Stock Standard** (see Prepartation of Standards)
    Dry Standard Target = 0.4394 g anhydrous potassium dihydrogen phosphate (KH_2PO_4), which has been dried at 110°C for 2 hours, to make 100 mg P/L stock solution.
    b. **Working Stock Standards and Working Standards**
    Working stock standards and working standards are made within a few days of the analysis (preferably the same day).
        1. Prepare working stock standards (usually 5000 and 1000 µg/L) from the stock standard.
        2. Prepare working standards from the working stock standards. Working standard concentrations should be in the range of expected sample concentrations (determined from past data). For example: 2, 5, 8, 10, 15, 25, 50, 75, 100, 150 µg/L
            a. Pick a target concentration (e.g. 1000 µg/L) and determine the mass of Stock Standard (or Working Stock Standard) needed to achieve the target (e.g. 1 g) (see chart in Preparation of Standards).
            b. Tare a 125 mL standards bottle and add the estimated mass of stock solution

c. Record the actual mass of stock used (e.g. 1.0387 g), and add enough Milli-Q to make approximately 100 g. Record the total mass (e.g. 99.5026 g).

d. Calculate the final concentration: (actual mass / total mass)*****stock concentration

(e.g. (1.0387g / 99.5026g) ***** 100327µg/L = 1047.31µg/L).

Example:

Target Conc.	**Est. Stock Wt.**	**Act. Stock Wt.**	**Total Wt.**	**Stock Conc.**	**Final Conc.**
1000 µg/L	1 g	1.0387 g	99.5026 g	100327 µg/L	1047.31 µg/L

6. **Make Reagents**

A. **STOCK AMMONIUM MOLYBDATE SOLUTION:** Combine 40 g **ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}*4H_2O$] and 983 g **Milli-Q**. Stir or shake until dissolved. Store in Nalgene bottle and refrigerate.

B. **STOCK ANTIMONY POTASSIUM TARTRATE SOLUTION:** Combine 3.0 g **antimony potassium tartrate** [$K(SbO)C_4H_4O_6*½H_2O$] and 995 g **Milli-Q**. Store in **dark** Nalgene bottle and refrigerate.

C. **MOLYBDATE COLOR REAGENT:** To a tared 1L dark container, add 680 g **Milli-Q**, then 64.4 g (approx. 35 mL) concentrated **sulfuric acid** (H_2SO_4) (Caution: the solution will get very hot!). Swirl to mix. When it can be handled comfortably, add 213 g stock **ammonium molybdate solution** (step A.) and 72 g stock **antimony potassium tartrate solution** (step B.). Shake and degas with helium before using.

D. **ASCORBIC ACID REDUCING SOLUTION**: Combine 60 g **ascorbic acid** and 975 g **Milli-Q**. Stir or shake until dissolved. Degas with helium. Add 1.0 g **dodecyl sodium sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Once DSS is added, the solution should never again be degassed. **Prepare fresh weekly**. For reduced quantities, follow the table below:

Ascorbic Acid	Milli-Q	Total Mass	DSS (after degassing)
60 g	975 g	1035 g	1.0 g
30 g	487.5 g	517.5 g	0.5 g
15 g	243.75	258.75 g	0.25 g

E. **CARRIER: Milli-Q**

F. **SODIUM HYDROXIDE - EDTA RINSE:** Dissolve 65 g **sodium hydroxide** and 6 g **tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in 1L or 1000 g Milli-Q.

7. **Enter Standard Concentrations and Sample ID's into Computer:**
Enter standard concentrations in descending order and establish boundaries of the segments with at least one standard between two

boundaries. Then enter sample ID's in the Sample Menu (see Appendix IV).

8. **Degas All Reagents** (including carrier) with helium for 2 minutes using appropriately labeled wands.
9. **Run Reagents Through Autoanalyzer** Snap cassettes onto pump with lines in place and arrows on cassettes pointing towards manifold. Rinse all manifold lines with Milli-Q before putting them in reagents. Cover reagents with parafilm and tape lines to bottles. Turn pump on "normal" speed (**NOTE** Dial next to pump should be set at 35). Check to be sure that reagents are not leaking and flowing freely by following air bubbles through individual lines. Be certain that there is enough of each reagent for the entire run. If additional reagents are added (with the exception of Milli-Q), a new calibration should be performed (see 12.).
10. **Download Method** (see Appendix IV). Baseline should be steady. An unsteady baseline indicates air in the reagents; degas them again.
11. **Pour Standards** into scintillation vials and place them in the standard rack in descending order.
12. **Calibrate Autoanalyzer**; i.e. run standards (see Appendix IV).
    a. If calibration is approved, start running samples.
    b. If calibration is not approved, look at calibration statistics and decide to either:
        1. Approve calibration (see Appendix IV).
        2. Redo calibration curve (perhaps with new reagents and/or new standards). See Trouble Shooting.
13. **Pour and Run Samples** (assuming that calibration has been approved). Pour samples into 13*100 mm tubes and insert them in the sampler rack. Place the rack in the position specified in the method. Submit the samples (see Appendix IV).
14. **Check Sample Data** and rerun samples if necessary
15. **Save the Data on Disc**. To copy only report data, see Appendix IV. To copy all raw data including the calibration curve, use the following DOS command:

    C:> COPY C:\LACHAT\METHODS\"method name"'\"file date"*.* A:

    For example:

    C:> COPY C:\LACHAT\METHODS\NEWTP2\940406*.* A:

    NOTE To reenter the Lachat menu from DOS, type "menu", or "mm" when in the subdirectory C:\LACHA

16. **Shut Down Manifold**
    a. Place ascorbic acid and color reagent lines in NaOH/EDTA rinse and pump for five minutes.
    b. Place all lines in Milli-Q (do not use this Milli-Q for anything other than flushing) and pump for five minutes
    c. Pump all lines dry.
17. **Shut Down/Clean-Up**
    a. Release manifold cassettes from pump.
    b. Put manifold lines in plastic bag
    c. Empty waste.
    d Turn off helium.
    e. Turn off fans.
    f. Turn off autoanalyzer.
    g. Soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.
    h. discard samples or store samples in freezer.

NITRATE

Method No. (10-107-04-1-B)
Range: 0.5-100µg/L
Method Name: NEWNO3-1 (pilot project NO3 on ch 1))

****NOTE**Check Method No. in Lachat Manual for most current method in use. If Method No. is different than that listed above, follow instructions in Lachat Manual and determine the current method name.**

Chemicals:

Sodium hydroxide (NaOH)
Ammonium chloride (NH_4Cl)
Disodium ethylenediamine tetraacetic acid dihydrate ($Na_2EDTA*2H_2O$)
85% phosphoric acid (H_3PO_4)
Sulfanilimide ($4\text{-}NH_2C_6H_4SO_2NH_2$)
N-(1-naphthyl)ethylenediamine dihydrochloride (NED)
Cadmium-copper reduction column (prepared by Lachat)
Potassium nitrate (KNO_3) (standard)
Chloroform ($CHCl_3$)

Analysis Instructions:

1. **Thaw samples**.
2. **Acid Wash** items necessary for analysis.**NOTE** Both fans should be turned on throughout entire procedure except when using the balance (0.1 mg sensitivity).
3. **Turn on Autoanalyzer** (heater should warm up for 45 min.), and inspect initial screen (look for large cased OK's)
4. **Install Manifold** on Channel 1. Refer to the diagrams on the wall to the left of the sampler to make sure that the manifold is correctly connected to the AE unit. In particular, pay attention to the diagram describing the connection and disconnection of the carrier line to the valve.**NOTE** Make sure the sample line is connected to the valve on the correct channel.
5. **Install Cadmium Reduction Column:**
   - A. Start pump and allow reagents to pump through system.
   - B. Make sure no air bubbles are in the lines. Connect the reduction column to the manifold loop according to the manifold diagram, taking care to avoid introducing air bubbles into the column.
   - C. Do not shut off the pump during this procedure.
   - D. If it is necessary to kill the run because there is some air in the line; first, disconnect the cadmium column and then pump reagents through all lines to expel any air. Finally, reconnect the cadmium column.
6. **Make Standards**:
   - a. **Stock Standard** (see Prepartation of Standards)
     Dry Standard Target = 0.7235 g potassium nitrate (KNO_3), which has been dried at 110°C for 2 hours,to make 100 mg N/L stock solution.
     ****NOTE**** Add 2 mL chloroform to volumetric flask after dissolving standard but before diluting to the mark. Stock standard is stable for 6 months
   - b. **Working Stock Standards and Working Standards**
     Working stock standards and working standards are made within a few days of the analysis (preferably the same day).
     1. Prepare working stock standards (usually 5000 and 1000 µg/L) from the stock standard.
     2. Prepare working standards from the working stock standards. Working standard concentrations should be in the range of expected sample concentrations (determined from past data). For example: 2, 5, 8, 10, 15, 25, 50, 75, 100 µg/L
        - a. Pick a target concentration (e.g. 1000 µg/L) and determine the mass of Stock Standard (or Working Stock Standard) needed to achieve the target (e.g. 1 g) (see chart in Preparation of Standards).

b. Tare a 125 mL standards bottle and add the estimated mass of stock solution

c. Record the actual mass of stock used (e.g. 1.0387 g), and add enough Milli-Q to make approximately 100 g. Record the total mass (e.g. 99.5026 g).

d. Calculate the final concentration: (actual mass / total mass)*****stock concentration

(e.g. (1.0387g / 99.5026g) ***** 100327µg/L = 1047.31µg/L).

Example:

Target Conc.	**Est. Stock Wt.**	**Act. Stock Wt.**	**Total Wt.**	**Stock Conc.**	**Final Conc.**
1000 µg/L	1 g	1.0387 g	99.5026 g	100327 µg/L	1047.31 µg/L

7. **Make Reagents**

A. **15M SODIUM HYDROXIDE (NAOH)**: Add 150 g **NaOH** slowly to 250 mL **Milli-Q**. Caution: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

B. **AMMONIUM CHLORIDE BUFFER, PH 8.5:** Combine 800 g **Milli-Q**, 126 g (approx. 105 mL) concentrated **HCl**, 85 g (approx. 95 mL) **ammonium hydroxide** (NH_4OH), and 1.0 g **disodium EDTA** (final mass = 1012 g). Mix well. Adjust pH up to 8.5 with 15M **sodium hydroxide** (from Step 1.) if necessary (it usually is not necessary). Make this reagent under the fume hood.

C. **SULFANILIMIDE COLOR REAGENT:** Combine 876 g **Milli-Q**, 170 g 85% **phosphoric acid** (H_3PO_4), 40.0 g **sulfanilimide**, and 1.0 g **N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake until wetted and stir with stir bar for 20 min. until dissolved. **Store in dark container. Stable for one month**. For reduced quantities follow the table below:

Milli-Q	Phos. Acid	Sulfanilimide	NED	Total Mass
876 g	170 g	40.0 g	1.0 g	1087 g
438 g	85 g	20.0 g	0.5 g	543.5 g
219 g	42.5 g	10.0 g	0.25 g	271.75 g

D. **CARRIER: Milli-Q**

8. **Enter Standard Concentrations and Sample ID's into Computer:** Enter standard concentrations in descending order and establish boundaries of the segments with at least one standard between two boundaries. Then enter sample ID's in the Sample Menu (see Appendix IV).

9. **Run Reagents Through Autoanalyzer** Snap cassettes onto pump with lines in place and arrows on cassettes pointing towards manifold. Rinse all manifold lines with Milli-Q before putting them in reagents. Cover reagents with parafilm and tape lines to bottles. Turn pump on "normal" speed (**NOTE** Dial next to pump should be set at 35). Check to be sure that reagents are not leaking and flowing freely by following air

bubbles through individual lines. Be certain that there is enough of each reagent for the entire run. If additional reagents are added (with the exception of Milli-Q), a new calibration should be performed (see 12.).

10. **Download Method** (see Appendix IV). Baseline should be steady. An unsteady baseline indicates air in the reagents; degas them again.
11. **Pour Standards** into scintillation vials and place them in the standard rack in descending order.
12. **Calibrate Autoanalyzer**; i.e. run standards (see Appendix IV).
    a. If calibration is approved, start running samples.
    b. If calibration is not approved, look at calibration statistics and decide to either:
        1. Approve calibration (see Appendix IV).
        2. Redo calibration curve (perhaps with new reagents and/or new standards). See Trouble Shooting.
13. **Pour and Run Samples** (assuming that calibration has been approved). Pour samples into 13*100 mm tubes and insert them in the sampler rack. Place the rack in the position specified in the method. Submit the samples (see Appendix IV).
14. **Check Sample Data** and rerun samples if necessary

15. **Save the Data on Disc**. To copy only report data, see Appendix IV. To copy all raw data including the calibration curve, use the following DOS command:

    C:> COPY C:\LACHAT\METHODS\"method name"'\"file date"*.* A:

    For example:

    C:> COPY C:\LACHAT\METHODS\NEWTP2\940406*.* A:

    NOTE To reenter the Lachat menu from DOS, type "menu", or "mm" when in the subdirectory C:\LACHAT.
16. **Shut Down Manifold**
    a. Stop the pump. Disconnect the cadmium column from the manifold loop and connect the ends with a union, taking care to avoid introduction of air bubbles.
    b. Restart the pump and place all feed lines in Milli-Q (do not use this Milli-Q for anything other than flushing) and pump for five minutes.
    c. Pump all lines dry.
17. **Shut Down/Clean-Up**
    a. Release manifold cassettes from pump.
    b. Put manifold lines in plastic bag
    c. Empty waste.
    d Turn off helium.
    e. Turn off fans.
    f. Turn off autoanalyzer.

g. Soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.
h. discard samples or store samples in freezer.

Total Nutrients

TOTAL PHOSPHORUS

Method No. (10-115-01-1-F)
Range: 1.0-200 µg/L
Method Name: NEWTP2
Method in use since 7/93

****NOTE**Check Method No. in Lachat Manual for most current method in use. If Method No. is different than that listed above, follow instructions in Lachat Manual and determine the current method name.**

Additional Equipment and Supplies:

16 x 100 mm tubes with plastic caps, acid washed
Autoclave
Several 400 - 600 mL beakers

Chemicals:

H_2SO_4
Ammonium molybdate tetrahydrate $[(NH_4)Mo_7O_{24}*4H_2O]$
Antimony Potassium tartrate $[K(SbO)C_4H_4O_6* ½ H_2O]$
Ascorbic Acid
Sodium hydroxide (NaOH)
Tetrasodium ethylenediamine tetraacetic acid (Na_4EDTA)
Anhydrous potassium dihydrogen phosphate (KH_2PO_4) (standard)
Ammonium persulfate ($(NH_4)_2S_2O_8$)
Adenosine monophosphate (AMP) (test standard)

Analysis Instructions:

1. **Thaw samples**.
2. **Acid Wash** items necessary for analysis.**NOTE** Both fans should be turned on throughout entire procedure except when using the balance (0.1 mg sensitivity).

3. **Turn on Autoanalyzer** (heater should warm up for 45 min.), and inspect initial screen (look for large cased OK's)
4. **Install Manifold** on Channel 2. Refer to the diagrams on the wall to the left of the sampler to make sure that the manifold is correctly connected to the AE unit. In particular, pay attention to the diagram describing the connection and disconnection of the carrier line to the valve.**NOTE** Make sure the sample line is connected to the valve on the correct channel.
5. **Make Standards**:
   a. **Stock Standard** (see Prepartation of Standards)
   Dry Standard Target = 0.4394 g anhydrous potassium dihydrogen phosphate (KH_2PO_4), which has been dried at 110°C for 2 hours, to make 100 mg P/L stock solution.
   b. **Working Stock Standards and Working Standards**
   Working stock standards and working standards are made within a few days of the analysis (preferably the same day).
      1. Prepare working stock standards (usually 5000 and 1000 µg/L) from the stock standard.
      2. Prepare working standards from the working stock standards. Working standard concentrations should be in the range of expected sample concentrations (determined from past data). For example: 2, 5, 8, 10, 15, 25, 50, 75, 100, 150, 1000 µg/L
         a. Pick a target concentration (e.g. 1000 µg/L) and determine the mass of Stock Standard (or Working Stock Standard) needed to achieve the target (e.g. 1 g) (see chart in Peparation of Standards).
         b. Tare a 125 mL standards bottle and add the estimated mass of stock solution
         c. Record the actual mass of stock used (e.g. 1.0387 g), and add enough Milli-Q to make approximately 100 g. Record the total mass (e.g. 99.5026 g).
         d. Calculate the final concentration: (actual mass / total mass)*****stock concentration

      (e.g. (1.0387g / 99.5026g) ***** 100327µg/L = 1047.31µg/L).

Example:

Target Conc.	Est. Stock Wt.	Act. Stock Wt.	Total Wt.	Stock Conc.	Final Conc.
1000 µg/L	1 g	1.0387 g	99.5026 g	100327 µg/L	1047.31 µg/L

   c. **Test Standards**
   Use Adenosine monophosphate (AMP) ($C_{10}H_{14}N_5O_7P$) to determine completeness of digestion (see Preparation of Standards, target = 0.1121 g, AMP/L = 10 mg P/L). Make test standards in the range of expected sample concentrations, e.g. 50 and 100 µg/L, and digest and run them with the samples.

6. **Digest Samples and Standards** **NOTE** Digestion method has been modified to accomodate equipement and supplies available at UNDERC.

A. Have ready:
   1. Freshly acid washed, dry digestion tubes (16*100 mm), and numbered screw on caps.
   2. Freshly (within a few days) acid washed 10 mL-size Oxford pipette tips.
   3. Freshly made standards (within 1-2 days) and reagents (ascorbic acid within 1 week).

B. Pipette **10 mL** of each of the following into a digestion tube using the labelled Oxford 5-10 mL pipettor and a different tip for each sample:
   1. Lake samples.
   2. **2** of **each** standard. (standards used to generate the standard curve must be processed (digested) in same manner as samples).
   3. 2-4 blanks (freshly drawn Milli-Q).
   4. 2 AMP (adenosine monophosphate) standards (e.g. 50 and 100 µg/L).

C. Add 1 mL ammonium persulfate digestion solution to each sample.

D. Screw on cap tightly (some loosen in autoclave so make sure they are on tight) and invert each sample several times to mix.

E. Place tubes in beakers and place beakers in autoclave. Be sure to add water to autoclave and heat tubes at 250 °F for at least 25 minutes (Note: UDERC autoclave can fit approx. 100 tubes, and takes approx 30 minutes to warm up to 250 °F.)

F. Once the temperature of the autoclave has decreased enough to be safely opened, remove the tubes and let them cool until they are near room temperature. Tubes can be cooled in tap water (be cautious that the tubes don't break as a result of the temperature difference between the tubes and the water).

G. Transfer one set of standards to scintillation vials and place in Lachat standards rack. Keep second set of standards in reserve in case they are needed. Process standards to generate calibration curve (see Appendix IV).

H. Once the calibration is running smoothly, transfer the samples to Lachat sample tubes (disposable, 13*100 mm borosilicate tubes). After the calibration is approved, submit the samples for analysis (see Appendix IV).

7. **Make Reagents**

a. **AMMONIUM PERSULFATE DIGESTION SOLUTION:** Combine 40 g **ammonium persulfate** and 480 g **Milli-Q** or follow recipes on chart:

Ammon. persulfate	Milli-Q	Total Mass
40 g	480 g	520 g
20 g	240 g	260 g
8 g	96 g	104 g

b. **0.11 M SULFURIC ACID CARRIER SOLUTION:** Combine 994 g **Milli-Q** and 11.24 g **concentrated sulfuric acid** (total mass = 1005.24 g)

c. **STOCK AMMONIUM MOLYBDATE SOLUTION:** Combine 40 g **ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24}*4H_2O]$ and 983 g **Milli-Q**. Stir or shake until dissolved. Store in Nalgene bottle and refrigerate.

d. **STOCK ANTIMONY POTASSIUM TARTRATE SOLUTION:** Combine 3.0 g **antimony potassium tartrate** $[K(SbO)C_4H_4O_6*{}^1/_2H_2O]$ and 995 g **Milli-Q**. Store in **dark** Nalgene bottle and refrigerate.

e. **MOLYBDATE COLOR REAGENT:** To a tared 1L **dark** bottle, add 694 g **Milli-Q**, then 38.4 g (approx. 20 mL) concentrated **sulfuric acid** (H_2SO_4) (Caution: the solution will get very hot!). Swirl to mix. When it can be handled comfortably, add 213 g **stock ammonium molybdate solution** (step a.) and 72 g **stock antimony potassium tartrate solution** (step b.). Shake and degas with helium before using.

f. **ASCORBIC ACID REDUCING SOLUTION**: Combine 60 g **ascorbic acid** and 975 g **Milli-Q**. Stir or shake until dissolved. Degas with helium. Add 1.0 g **dodecyl sodium sulfate** $(CH_3(CH_2)_{11}OSO_3Na$. Once DSS is added, the solution should never again be degassed. **Prepare fresh weekly**. For reduced quantities, follow the table below:

Ascorbic Acid	Milli-Q	Total Mass	DSS (after degassing)
60 g	975 g	1035 g	1.0 g
30 g	487.5 g	517.5 g	0.5 g
15 g	243.75	258.75 g	0.25 g

g. **SODIUM HYDROXIDE - EDTA RINSE:** Dissolve 65 g **sodium hydroxide** and 6 g **tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in 1L or 1000 g **Milli-Q**.

8. **Enter Standard Concentrations and Sample ID's into Computer:** Enter standard concentrations in descending order and establish boundaries of the segments with at least one standard between two boundaries. Then enter sample ID's in the Sample Menu (see Appendix IV).

9. **Degas All Reagents** (including carrier) with helium for 2 minutes using appropriately labeled wands. ****NOTE****Do not degas ascorbic acid after adding DSS.
10. **Run Reagents Through Autoanalyzer** Snap cassettes onto pump with lines in place and arrows on cassettes pointing towards manifold. Rinse all manifold lines with Milli-Q before putting them in reagents. Cover reagents with parafilm and tape lines to bottles. Turn pump on "normal" speed (**NOTE** Dial next to pump should be set at 35). Check to be sure that reagents are not leaking and flowing freely by following air bubbles through individual lines. Be certain that there is enough of each reagent for the entire run. If additional reagents are added (with the exception of Milli-Q), a new calibration should be performed (see 13.).
11. **Download Method** (see Appendix IV). Baseline should be steady. An unsteady baseline indicates air in the reagents; degas them again.
12. **Pour Standards** into scintillation vials and place them in the standard rack in descending order.
13. **Calibrate Autoanalyzer**; i.e. run standards (see Appendix IV).
    a. If calibration is approved, start running samples.
    b. If calibration is not approved, look at calibration statistics and decide to either:
        1. Approve calibration (see Appendix IV).
        2. Redo calibration curve (perhaps with new reagents and/or new standards). See Trouble Shooting.
14. **Pour and Run Samples** (assuming that calibration has been approved). Pour samples into 13*100 mm tubes and insert them in the sampler rack. Place the rack in the position specified in the method. Submit the samples (see Appendix IV).
15. **Check Sample Data** and rerun samples if necessary
16. **Save the Data on Disc**. To copy only report data, see Appendix IV. To copy all raw data including the calibration curve, use the following DOS command:

    C:> COPY C:\LACHAT\METHODS\"method name"'\"file date"*.* A:

    For example:

    C:> COPY C:\LACHAT\METHODS\NEWTP2\940406*.* A:

    NOTE To reenter the Lachat menu from DOS, type "menu", or "mm" when in the subdirectory C:\LACHAT.

17. **Shut Down Manifold**
    a. Place ascorbic acid and color reagent lines in NaOH/EDTA rinse and pump for five minutes.
    b. Place all lines in Milli-Q (do not use this Milli-Q for anything other than flushing) and pump for five minutes
    c. Pump all lines dry.

18. **Shut Down/Clean-Up**
    a. Release manifold cassettes from pump.
    b. Put manifold lines in plastic bag
    c. Empty waste.
    d Turn off helium.
    e. Turn off fans.
    f. Turn off autoanalyzer.
    g. Soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.
    h. discard samples or store samples in freezer.

TOTAL NITROGEN (KJELDAHL)

Method No. (10-107-06-2-E)
Range: 100-5000 µg/L
Method Name: NEWTKN1 (TKN ch. 1, 5/94)
Method in use since 5/94

****NOTE**Check Method No. in Lachat Manual for most current method in use. If Method No. is different than that listed above, follow instructions in Lachat Manual and determine the current method name.**

Additional Equipment and Supplies:

Block digestor
Digestion tubes for block digestor
Boiling chips, acid washed

Chemicals:

Sodium hydroxide (NaOH)
Sodium potassium tartrate (d,l-$NaKC_4H_4O_6*H_20$)
Sodium phosphate dibasic heptahydrate ($Na_2HPO_4*7H_2O$)
Sodium salicylate
    [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$]
Sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO*2H_20$
Regular Chlorox Bleach (5.25% sodium hypochlorite)
Ammonium chloride (NH_4Cl) (standard)
Sulfuric acid (H_2SO_4)
Mercuric oxide (HgO)
Potassium sulfate (K_2SO_4)
Nicotinic acid (test standard)

Analysis Instructions:

1. **Thaw samples**.
2. **Acid Wash** items necessary for analysis.**NOTE** Both fans should be turned on throughout entire procedure except when using the balance (0.1 mg sensitivity).
3. **Turn on Autoanalyzer** (heater should warm up for 45 min.), and inspect initial screen (look for large cased OK's)
4. **Install Manifold**: Refer to the diagrams on the wall to the left of the sampler to make sure that the manifold is correctly connected to the AE unit. In particular, pay attention to the diagram describing the connection and disconnection of the carrier line to the valve.**NOTE** Make sure the sample line is connected to the valve on the correct channel.
5. **Make Standards**:
   a. **Stock Standard** (see Prepartation of Standards)
      Dry Standard Target: = 3.819 g ammonium chloride (NH_4Cl), which has been dried at 110°C for 2 hours, to make 1000 mg N/L stock solution or 0.3819 g NH_4Cl to make 100 mg N/L.
   b. **Working Stock Standards and Working Standards**
      Working stock standards and working standards are made within a few days of the analysis (preferably the same day).
      1. Prepare working stock standards (usually 5000 and 1000 µg/L) from the stock standard.
      2. Prepare working standards from the working stock standards. Working standard concentrations should be in the range of expected sample concentrations (determined from past data). For example: 50, 100, 200, 300, 400, 500, 1000 µg/L
         a. Pick a target concentration (e.g. 1000 µg/L) and determine the mass of Stock Standard (or Working Stock Standard) needed to achieve the target (e.g. 1 g) (see chart in Peparation of Standards).
         b. Tare a 125 mL standards bottle and add the estimated mass of stock solution
         c. Record the actual mass of stock used (e.g. 1.0387 g), and add enough Milli-Q to make approximately 100 g. Record the total mass (e.g. 99.5026 g).
         d. Calculate the final concentration: (actual mass / total mass)*****stock concentration

         (e.g. (1.0387g / 99.5026g) ***** 100327µg/L = 1047.31µg/L).

Example:

Target Conc.	Est. Stock Wt.	Act. Stock Wt.	Total Wt.	Stock Conc.	Final Conc.
1000 µg/L	1 g	1.0387 g	99.5026 g	100327 µg/L	1047.31 µg/L

c. **Test standards**
Use Nicotinic acid ($C_6H_5NO_2$) to determine completeness of digestion. (see Preparation of Standards, target = 0.8787 g nicotinic acid/L = 100 mg N/L). Make test standards in the range of expected sample concentrations, e.g 200 and 500 µg/L, and digest and run them with the samples.

6. **Digest Samples and Standards****NOTE** Digestion method has been modified to accomodate equipement and supplies available at UNDERC
   a. Preheat block digestor (under hood) to 160°C. It should be preheated at least 30 minutes prior to use.
   b. Have ready: (fresh means within 1-2 days unless otherwise noted)
      1. freshly acid washed 75 mL digestion tubes placed in block digestor rack.
      2. freshly (just before use) acid washed Teflon boiling chips
      3. freshly acid washed tips for Oxford 5-10 mL pipettor
   c. Pipette **15 mL** (2 x 7.5 mL) of each of the following into a digestion tube, using the labeled Oxford 5-10 ml pipettor and a different tip for each sample:
      1. Lake samples.
      2. Each standard (standards used to generate the standard curve must be processed (digested) in same manner as samples).
      3. 2-4 blanks (freshly drawn Milli-Q).
      4. 2 nicotinic acid test standards (e.g. 200 and 500 µg/L).
   d. Add **2 mL** TN digestion solution to each tube.
   e. Add 4-5 acid washed Teflon boiling chips to each tube.
   f. Mix well with vortex mixer.
   g. Place rack with sample tubes in digestor. **Be sure to attach side panels to rack before digesting.**
   h. Turn on hood and lower hood window to proper level.
   i. Digest samples at 160°C for 75 min then at 380°C for 75 min. Use automatic dual setting on block digestor. Light on digestor indicates whether temperature has been reached.
   j. After samples are digested, lift rack out of the block digestor and rest it on bricks under hood until samples stop fuming. ****NOTE** Take precautions to avoid breathing in the fumes.**
   k. When samples are fairly cool, pipette **15 mL** freshly dispensed Milli-Q into each tube. Mix very well (at least 15 sec. per sample) using vortex mixer. Check to be sure that residue in tube is completely dissolved.

7. **Make Reagents**

A. **STOCK MERCURIC SULFATE SOLUTION**: To a 100 mL volumetric flask, add approximately 40 mL **Milli-Q** and 10 mL **concentrated sulfuric acid** (H_2SO_4). Then add 8.0 g **red mercuric oxide** (HgO). Stir until dissolved, dilute to mark and mix well.

B. **DIGESTION SOLUTION:** Add approximately 200 ml to a 500mL volumetric flask. Then add 66.5 g **potassium sulfate** (K_2SO_4) and 100 mL **concentrated sulfuric acid** (H_2SO_4). Add 12.5 mL **stock mercuric sulfate solution** (see A.). Dilute to the mark with Milli-Q and mix well. Make fresh monthly. (This is the recipe for a half batch and this is all that is usually necessary.)

C. **BUFFER:** Completely dissolve 35 g **sodium phosphate dibasic heptahydrate** ($Na_2HPO_4*7H_2O$) in 934 g **Milli-Q**. Add 20 g **disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 65 g **sodium hydroxide** (NaOH). Shake until dissolved

D. **SALICYLATE-NITROPRUSSIDE COLOR REAGENT: In a dark Nalgene bottle,** combine 150 g **sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], 1.00 g **sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO*2H_2O$] and 908 g **Milli-Q** (for a final mass of 1059 g). Shake or stir until dissolved. **Store . Prepare at least weekly.** For reduced quantities, follow the table below:

Salicylate	Nitroprusside	Milli-Q	Total mass
150 g	1.00 g	908 g	1059 g
75g	0.5 g	454	529.5 g
37.5g	0.25 g	227 g	264.75 g

E. **HYPOCHLORITE REAGENT**: Combine 32 g regular **Chlorox Bleach** (5.25%sodium hypochlorite) and 468 g **Milli-Q** (for final mass of 500 g). Mix well.

8. **Enter Standard Concentrations and Sample ID's into Computer:** Enter standard concentrations in descending order and establish boundaries of the segments with at least one standard between two boundaries. Then enter sample ID's in the Sample Menu (see Appendix IV).

9. **Degas All Reagents** (including carrier) with helium for 2 minutes using appropriately labeled wands. Cover reagents with parafilm.

10. **Run Reagents Through Autoanalyzer** Snap cassettes onto pump with lines in place and arrows on cassettes pointing towards manifold.

a. Place all manifold lines in Milli-Q. Turn pump on "normal" speed (**NOTE** Dial next to pump should be set at 35). Check to be sure lines are not leaking and flowing freely by following air bubbles through individual lines

b. Place the buffer line in the buffer and pump until the air bubble introduced reaches the "T" fitting on the manifold. Then place all other lines in the proper containers. Be certain that there is enough of each reagent for the entire run. If additional reagents are added (with the exception of Milli-Q), a new calibration should be run (see 13.).

11. **Download Method** (see Appendix IV). Baseline should be steady. An unsteady baseline indicates air in the reagents; degas them again.

12. **Pour Standards** into scintillation vials and place them in the standard rack in descending order.

13. **Calibrate Autoanalyzer**; i.e. run standards (see Appendix IV).
    a. If calibration is approved, start running samples.
    b. If calibration is not approved, look at calibration statistics and decide to either:
        1. Approve calibration (see Appendix IV).
        2. Redo calibration curve (perhaps with new reagents and/or new standards). See Trouble Shooting.

14. **Pour and Run Samples** (assuming that calibration has been approved). Pour samples into 13*100 mm tubes and insert them in the sampler rack. Place the rack in the position specified in the method. Submit the samples (see Appendix IV).

15. **Check Sample Data** and rerun samples if necessary

16. **Save the Data on Disc**. To copy only report data, see Appendix IV. To copy all raw data including the calibration curve, use the following DOS command:

    C:> COPY C:\LACHAT\METHODS\"method name"'\"file date"*.* A:

    For example:

    C:> COPY C:\LACHAT\METHODS\NEWTP2\940406*.* A:

    NOTE To reenter the Lachat menu from DOS, type "menu", or "mm" when in the subdirectory C:\LACHAT.

17. **Shut Down Manifold**
    a. At the end of the run, place all lines **except buffer** in Milli-Q, and flush system for one or two minutes.
    b. Place buffer line in Milli-Q (do not use this Milli-Q for anything other than flushing), and pump for another two to three minutes.
    c. If a potential clog or precision problems (drifting baseline, wide peaks etc.) are suspected , run HCl (1 or 2 M) through all lines for 2-3 minutes. Then flush again with Milli-Q for 2-3 minutes.
    d. Pump all lines dry.

18. **Shut Down/Clean-Up**
    a. Release manifold cassettes from pump.
    b. Put manifold lines in plastic bag
    c. Empty waste.
    d Turn off helium.
    e. Turn off fans.
    f. Turn off autoanalyzer.
    g. Soak necessary items in acid wash (after rinsing with tap water): degassing wands; scint. vials; sample, standard, and reagent bottles; pipette tips; sample and digestion tubes.
    h. discard samples or store samples in freezer.

REFERENCES:

Methods Manual for the QuikChem Automated Ion Analyzer Lachat Instruments

(sedtrap.doc)

SEDIMENT TRAP SAMPLING:

At the beginning of the sampling season, deep sediment traps are suspended from floats (3 traps per set, 2 sets) in each lake (Peter-12 m, Paul-9 m East Long-12 m, West Long-12 m), and are retrieved and sampled biweekly (alternate with PPR weeks).

1. To retrieve traps:

   a. Slide cover down rope, wait until it hits, then pull up trap.

   b. Pool the water from the three traps for each set into a 2 L jug, rinsing each trap well with distilled water.

   c. Store the jugs in the refrigerator until ready to process the samples.

2. Shake sample jug vigorously and empty into a 2L graduated cylinder. Measure and record the total volume of water for each set of traps.

3. Label two 125 mL sample bottles with "Sed. Trap", lake, range of days, and "TN" or "TP". For each trap, pour approximately 100 mL into each of the bottles.

CALCULATIONS:

To calculate sedimentation rate of P or N in sediment trap, use the following formula:

$$\text{P sediment} = \frac{([TP_{trap}] - [TP_{hypo}]) * 0.507L}{0.00203m^2 * 14d}$$

where:

P sediment = sedimentation rate in $\mu g * m^{-2} * day^{-1}$

TP_{trap} = concentration of P (in µg/L) in the trap (data from Lachat autoanalyser)

TP_{hypo}= concentration of P (in µg/L) in the hypolimnion on the day of collection

0.507 L = volume of one cylinder of sediment trap

0.00203 m^2 = area sediment trap opening

14 days = days sediment trap is deployed

Use the analogous equation for N.

GRAN ALKALINITY METHOD FOR DETERMINATION OF DIC

Note: This method last used summer of 1995

EQUIPMENT:

10-100 µL automatic pipettor
100-1000 µL automatic pipettor
100 mL volumetric flask
Magnetic stirrer
pH meter, accuracy ± 0.01 pH units
6, 125 mL Erlenmeyer flasks

REAGENTS:

0.01N H_2SO_4

PROCEDURE:

0. Open cover on pH probe, and calibrate pH meter (see pH meter instructions)

1. Obtain 100 mL of sample (V_s) and place in a 125-mL flask.

    a. Samples include: 1% surface irradiance; either 5% or 10% of S.I. (whichever sample best represents the metalimnion); and PML (pooled mixed layer).

2. Stir sample for 10 minutes with pH electrode in place. pH should stabilize.

3. Record initial pH and temperature.

4. Titrate the water sample to pH 4.5 with 25 or 50 µL acid additions, and record volume of acid added (V_a).

5. Titrate to pH 3.5 with 50 µL acid additions (more or less depending on lake and depth), maximizing the number of additions to obtain 15-20 points.

    a. Record the cumulative acid additions (V_a) and pH after each addition.

6. Rinse glassware with Milli-Q and dry on rack.

7. Close cover on pH probe

CALCULATIONS:

Use the CALCGRAN.CMD SYSTAT program to calculate alkalinity based on the following calculations:

1. Calculate for each acid addition between pH 4.5 and 3.5:

$$F1 = 10^{-pH}(V_s+V_a)$$

where V_s is sample volume in mL and V_a is cumulative acid volume in mL.

2. Regress F1 (ordinate) against V_a (abscissa) and calculate endpoint volume (V_e) as:

$$V_e = \frac{-1*a}{b}$$

a = intercept
b = slope

3. Alkalinity (A in μeqL^{-1}):

$$A = \frac{V_e * 10^6 * N}{V_s}$$

V_e = endpoint volume
N = acid normality
V_s = sample volume

4. DIC is calculated from a regression equation (see program for equation)

REFERENCES:

Gran, G. 1952. Determination of equivalence point in potentiometric titrations. Analyst, 77:661-671.

Zimmerman, A.P. and H.H. Harvey. 1979. Final report on sensitivity to acidification of waters of Ontario and neighboring states for Ontario Hydro. University of Toronto. pp. 6-14.

(gcproc97.do)

Gas Chromatograph Method for Measurement of PCO2 and DIC

EQUIPMENT:

50 mL syringes
Gas Chromatograph (Shimadzu GC-8AIT) fitted with:
- C-RA Recording Data Processor
- GC 8A Thermal Conductance Detector
- 1/8" 2.0M Stainless Steel Column packed with Porapak Q, 80/100
- 1/8" Column Adapter
- 3-way Valve and Adapter
- MGS-4 Gas Sampler and Adapter
- Gas regulators for He tanks
- Chart paper
- Soap film flow meter
- Pipette tip (at gas injection point) filled with drierite (10-20 mesh, color indicating) and cotton

100-1000 uL automatic pipettor (for acid injection)
CO_2 gas standards
- 1000 ppm
- 10000 ppm

2N H_2SO_4
$NaHCO_3$ Standard in Milli-Q (0.5, 1.0, 2.0, and 4.0 mg/L)
- 4.0 mg/L standard: 0.0280 g $NaHCO_3$ per liter of Milli-Q
- (shelf life of about one week.)

OVERVIEW:

The partial pressure of aqueous CO_2 (pCO_2) is the amount of free CO_2 in water. It is measured by equilibrating an "infinitely large" sample of water with an "infinitely small" sample of air and measuring the resulting CO_2 concentration in the air phase.

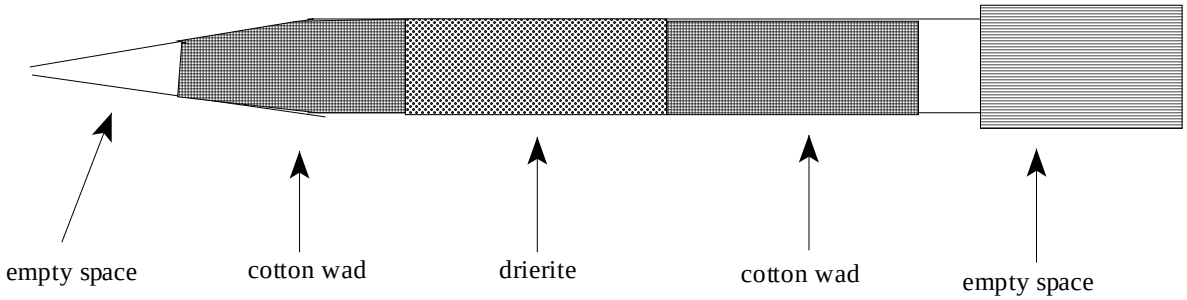
Dissolved Inorganic Carbon (DIC) is the sum of all the carbonate anions found in the water. Lake samples are acidified, causing all DIC to become CO_2. The acidified sample is injected into the gas chromatograph, which measures the amount of CO_2 against a known gas standard.

PROCEDURE:

Preparing the GC (Before going into the field)

1. **Check the color of the drierite in the tip** (where sample gas is injected into the GC.) If it is not entirely blue (i.e. the same color as when it was taken out of the jar), change the tip by lightly packing in some cotton into the end of a new 1 mL pipette tip, adding new drierite above the cotton, and finally adding more cotton above the drierite. The cotton should not be packed too tightly into the tip (a paperclip can be used for this purpose.) Reinstall the tip carefully being conscious of creating a good seal to prevent gas from escaping.

Tip for GC



NOTE: In order for the machine to run properly, it is important that these steps be done in the order given below

2. **Start the He flow on the GC helium tank**

Open the horizontal valve to the right of the regulator on the He tank connected to the GC. Do not move the other valves on the helium tank. The needles of the dials on the front of the GC (Carrier Gas 1 and 2) should move to the designated (red) marks. These marks have been calibrated for equal gas flow from the two carrier gases. If the needles stay at or near zero, make sure that the helium tank valve is really open. If the needles are near but not on the red marks, adjust them using the knobs on the side of the GC. Do not continue until the needles are on or near the red marks.

3. **Check the Settings**:
   a. **Column Temperature - 45 degrees**
   b. **Injection/Detection Temperature - 110 degrees**
   c. **Attenuation - 16**

4. **Warm up the GC** (approximately 30-60 minutes):
    a. **Turn on the GC** (switch is on the lower right)
    b. **Set the current to 160 mAmps** (turn up the current slowly, waiting a second or two between clicks)
    c. **Turn on the recorder** (switch is on the right back.) It will flash lights for several seconds and then the ready light will go on.

5. Check that the GC has warmed up

Check the level by entering 'PRINT LEVEL' (press 'PRINT' then simultaneously press 'CONTROL' and 'LEVEL' then 'ENTER') When the machine is cold, the level will be -5000. The level will drift towards zero. To check that the machine is warmed up, check the level every few minutes until there is little change. Adjust the level to a number close to zero using the FINE ZERO knob and recheck the level in a few minutes to be sure that the machine is properly warmed up. **Be sure not to adjust the level knobs once the analysis has begun.**

Running a GC Sample

Following are general steps for running a sample (use for procedures which follow)

a. Make sure that the dials on the Gas Sampler are set to **Charge, Close, Close**
b. **Open 1**
c. **Open 2**
d. **Inject the sample**
e. **Close 1**
f. **Wait 3 Seconds**
g. **Close 2**
h. Switch to **Discharge** (from Charge)
i. Press **Start** on the Recorder
j. Press **Stop** once the CO_2 peak has been seen on the chart to get a reading of its area

Checking for air leaks and for a CO_2 peak

1. Check for air leaks:

Inject a full syringe of lab air into the GC by following the steps below:

a. Prepare some soapy water and place it (using a finger or Pasteur pipette) at the connection where the tip fits onto the GC
b. Draw in a full syringe of lab air and insert it onto the tip
c. Follow the previous instructions for Running a GC Sample
d. While injecting the entire sample of lab air into the GC, look for air leaks at the tip-GC connection

e. If a leak is detected, reinstall the tip and repeat the above procedure until the problem is corrected.

2. Check to make sure a CO_2 peak is present

The first peak, which goes beyond the scale of chart, is a Nitrogen and Oxygen peak. This will soon be followed by a CO_2 peak (about 0.8 minutes after Discharge.) Once the CO_2 peak is completely seen on the chart, press **Stop** to get a readout of its area. Outside air is about 3500-4000 chart units. Lab air is usually similar to the outside air although Jon Cole has shown that if PI□s have been in the lab, the value could be as high as 10000 chart units.

Running Gas Standards

Once air leaks have been corrected, the 1000 ppm gas standard should be run
Note: If sample values are greater than 4 times the 1000 ppm CO_2 gas standard values, run the high gas standard (1% CO_2 or 10000 ppm)

1.Rinse (i.e. clear the air) from the syringe and the lines

a. With the syringe attached to the 1000 ppm standard gas cannister, quickly open and close the valve of the cannister
b. Open the valve of the syringe to allow for a small amount (approximately 5 mL) of gas standard to enter
c. Quickly close the valve and purge the standard (this will clear the air from the line)

2. Take the Gas Standard Sample

a. Reopen the valve of the syringe, allowing it to fill up with the gas standard
b. Withdraw the syringe from the gas cannister and quickly place a finger over the syringe tip
c. Connect the syringe to the GC tip

3. Inject the standard into the GC

a. Following the previous instructions for Running a GC sample, **inject 5 mL** of the standard into the GC
b. Wait for the Nitrogen/Oxygen peak (the first peak) to reach a maximum and switch the dials to **Open, Open, Charge**
c. Inject another 5 mL of standard into the GC (followed by **Close1, wait 3 seconds, Close 2, Discharge**)
d. Repeat this procedure until at least 4 standards are run or until the syringe is empty of standard.

If all of the standard values are within 5% of each other, proceed to running the samples. Typical standard gas values are about 10000-12000 chart units. If values are outside of this range, consult someone.

Running the pCO_2 samples:

The pCO_2 and field air samples should be run as soon as they are brought into the lab

1. Place a tissue on the end of the syringe, open the stopcock, expel a small amount of sample (to eliminate any water droplets at the end of the syringe), and quickly close the stopcock.
2. Attach the syringe to the GC tip and run the sample as explained in the previous instructions. Only **inject 10 mL** of sample into the GC (saving at least half of the sample in case it needs to be rerun)
3. Attach another syringe to the GC tip and inject 10 mL or so of the sample following the above directions for injecting a second sample (as seen in instructions for injecting the standards)
4. Wait for the water peak to run through before running the remaining 2 pCO_2 samples and the 2 field air samples. Note that unlike the standards, only 2 samples can be run consecutively after which it is necessary to wait for a water peak to run through (a water peak is not present when running standards.) The water peak takes a set amount of time before appearing and disappearing. Two more samples can be run approximately 5 minutes after the pCO_2 peak from the previous sample (for maximum efficiency, use a timer.)
5. If the 4 pCO_2 samples are not within 5% of each other, rerun the samples (or at least rerun the outliers)

Running the DIC samples

1. Preparing Samples for Injection
    a. Fill 3 syringes per depth with sample water from the 6 light depths as soon as possible after the water has been brought to the lab, before the sample water has been processed (i.e. shaken up) for chlorophyll filtering.
        1) Each syringe should be rinsed 3 times with sample before being filled
        2) After a syringe is filled, tap air bubbles to the tip and expel them, leaving at least 12 mL of liquid
    b. Acidify the Sample
        1) Reduce the volume of the sample to exactly 10 mL
        2) Inject 200 uL of 2N H_2SO_4 directly into the syringe using a pipette. The syringe plunger needs to be controlled while the acid is injected to both allow for the acid to enter the sample and to prevent air from entering the sample.
    c. Inject He into the sample (immediately after the acid has been added)
        1) Insert the syringe into the helium tank
        2) Turn the regulator until the syringe slowly begins to fill with helium

3) Turn off the helium flow just before the total volume in the syringe reaches 30 mL and stop helium injection into the syringe at exactly 30 mL total volume
4) Withdraw syringe from the helium tank and quickly cover the end with a finger
5) Cap syringe

d. Repeat acidification and helium injection with a second sample (Follow Steps 3 and 4)
e. Shake the 2 samples vigorously and simultaneously for 1 minute

2. Inject the Samples into the GC

Inject the 2 samples into the GC as outlined above (Running the pCO2 Samples)

a. Before attaching the syringe to the GC, be sure to inject a small amount of sample into a tissue to expel the water at the tip of the syringe
b. **Be extremely careful to not inject water into the GC tip** (keep the syringe tilted down when injecting the sample and quit injecting the sample well before the water is close to the GC tip; injecting 10 mL of gas sample is more than sufficient)

If replicate samples are within 5% of each other, proceed to the next sample depth

Rerunning The Gas Standard

Once all samples are run, the 1000 ppm CO2 gas standard should be rerun. Follow the steps outlined above (Running the Gas Standard). Compare the values with those obtained before running the samples. All values should be within 5% of each other.

Shutting down the GC

It is important to perform these steps in the proper order

a) Flush the GC with helium by injecting a syringe of helium into the GC
b) Slowly turn the Current to 0 (wait 1 second between clicks)
c) Turn off the Recorder
d) Turn off the GC
e) Shut off the helium flow of both tanks (only turn off the horizontal knob to the right of the regulator)

Saving DIC Samples

If the GC does not appear to be running correctly, DIC samples should be saved and rerun at a later date. In the past, samples have been saved in TIC/TOC Shimadzu tubes with snap on lids and run at IES. It appears that an air tight seal was not obtained (when the samples were run they had equilibrated with the air). Thus experimenting with the lid to obtain a good seal will be necessary. Until the tubes prove to be effective, samples should be saved in both the tubes and in

BOD bottles. Samples saved in BOD bottles can be run on the GC once the problem is fixed (thus they should definitely be used if the GC problem appears to be temporary.)

1.Saving the samples in the tubes

a. Fill the tube gently using a syringe. It may be necessary to leave a small amount of head space to obtain an air tight seal once the cap is put on.
b. Add 70 ul of 2N H_2SO_4 using a pipette (tube volume is 7.4 mL) and injecting the acid under the water level
c. Snap on the cap
d. Label the tube
e. Save $NaHCO_3$ liquid standards in the tubes using this same method. Save standards which are in the range of the sample concentrations (perhaps 500 ug/L and 1000 ug/L)

2.Saving the Samples in BOD Bottles

a. Add the water sample to the BOD bottle by dipping it under the water level. To save space, use the smallest BOD bottle available (60mL)
b. Add 200 ul of 2N H_2SO_4 for each 10 ml of sample (this is the same ratio of acid added to the DIC samples.) Note: Acid should always be added if the sample may be stored for a week or longer before being analyzed.
c. Cap tightly with a BOD stopper

3. Running the samples in BOD bottles

a. Take the replicate samples from the BOD bottle using a syringe with a piece of tubing attached to the end. Try to introduce as little air as possible.
b. Process the samples as outlined above (Running the DIC Samples.) Remember not to acidify the sample if acid has already been added to the BOD bottle

<u>Periodic GC Checks and Maintenance</u>

1.Checking for Gas Leaks

Gas leaks in the GC should be checked for at the beginning of and perhaps periodically throughout the field season. Use soapy water to check for leaks at connections in tubing between the helium tank and the GC and tubing behind where the sample is injected (a Pasteur pipette can be used to distribute the soap). The most critical leaks are those in the tubing located behind where the sample is injected (i.e. internal to the GC.) When checking for these leaks, make sure the proper valves are open, allowing gas to flow through. The leaks from the helium tank should not effect the operation of the GC but they do account for waste of expensive helium.

2. Running Liquid Standards

Liquid standards should be run periodically. The advantage of using a liquid standard is that it is run exactly like a liquid DIC sample and thus suspected problems with acidification or other sample processing procedures can be checked. However, the accuracy of the gas standard should be better. If the values obtained when running the gas standards are fairly constant on a daily basis and are not changing much over the field season, and other problems with the DIC samples are not suspected, liquid standards may only need to be run a few times during the field season.

a. Dissolve $NaHCO_3$ in distilled water to make dilutions of 0.0, 0.5, 1.0, 2.0, and 4.0 mg/L, and process them just like DIC samples but do not run them in duplicate. A dilution of 1.0 g/L can be stored in the refrigerator during the field season and the above dilutions can be periodically made from it.
b. Compare the expected values with the values obtained when calibrated from the gas standard
c. Perhaps run a linear regression of the expected concentrations vs. the area values (i.e. chart units) and check the r^2 value. An r^2 value of .98 is satisfactory.

3. Calibrating Gas Flow

This may only have to be done once a field season.

a. Once the machine is turned on plug the open end of the soap film flow meter into the port 1.
b. Squeeze the soap from the plastic tube at the bottom of the flow meter until the gas forces it up the glass tube. Once the glass tube is lubricated with soap, time how long it takes for a soap bubble to move a designated distance (marked in mL.) In the past we have used a flow rate of 40 mL/minute. To change the flow rate, move the appropriate knob on the side of the machine.
c. Repeat steps a and b for the other channel (i.e. port 2). Adjust the flow rate so that the two channels have an equal rate.
d. Mark the position of the needles on the dials on the front of the GC. Subsequently, when the GC is turned on, the needles should move to the marked positions. If not, the proper adjustments should be made (using the knobs on the side of the machine.) The needles moving to the marks indicates that gas flow from the two channels is the same and has remained constant. However, if the column is plugged up, it is possible that the readings on the dial will falsely indicate the predetermined gas flow. Thus checking the gas flow when GC problems occur is a good troubleshooting step.

Troubleshooting

1. Connection Problems between the GC and the Recorder

Occasionally the recorder does not communicate with the GC (this problem is detected when there is no response to changing the Fine Zero knob.) In the past, this problem has been corrected by turning the GC and Recorder off and then back on, carefully following the correct order for doing this, as outlined above (Note: it is not necessary to turn off the helium.)

Reference:

Stainton, M.P., M.J. Capal and F.A.J. Armstrong. 1997. The Chemical Analysis of Fresh Water. 2nd edition. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 166p.

(bacabun97.doc)

MEASUREMENT OF BACTERIAL ABUNDANCE

EQUIPMENT:

0.8 (or .45) µm x 25 mm Millipore filters (backing filters)
0.2 µm x 25 mm irgalan black stained Nuclepore polycarbonate filters
Slides and cover slips
syringe (≥10 cc)
0.2 µm x 25 mm Millipore filters and Swinnex or 0.2 µm x 25 mm Gelman sterile Acrodiscs
Filtration manifold
Filtration towers
Vacuum pump with tubing
100-1000 µl pipettor

REAGENTS:

2% formaldehyde, 0.2 µm filtered (5 ml 37% formaldehyde + 90 ml distilled water)
0.05% acridine orange
Cargille type A immersion oil
Distilled water

PROCEDURE:

A. Preparation of unstained polycarbonate filters
(Only necessary with filters not pre-stained)

Add 0.2 g Irgalan black to 2% acetic acid (2 ml glacial acetic acid + 98 ml distilled water). This does not need to be filtered. Immerse filters in Irgalan black solution for 1-2 hours. Carefully pour off solution (save and re-use). Rinse filters several times with distilled water. Filters may be stored in distilled water or air-dried on paper towels and then stored.

B. Preparation of acridine orange

Make 0.05% solution by adding 50 mg acridine orange to 100 ml distilled water. Filter using a syringe and 0.2 µm Acrodisc or swinnex filter holder with a 0.2 µm Millipore filter. Freeze 10 ml aliquots in plastic scintillation vials.

C. Slide preparation

Slides should be made as soon as possible after sample collection. Take care not to cross-contaminate reagents and samples with pipet tips.

1. Place wetted backing filters and stained Nuclepore filters on filtration towers.

2. Gently stir the sample and remove 1 ml with a pipet. Add the sample to the filtration tower and dilute up to 2 ml with filtered 2% formaldehyde (more sample should be added if filters do not show at least 40 cells per grid.)

3. Add 0.2 ml acridine orange and stain for 2 minutes.

4. Filter samples at <200 mm Hg vacuum pressure.

5. Remove filters, allow to air dry, place on a labeled (lake, date, depth, volume filtered, filter tower used) slide between two drops of immersion oil and cover with a cover slip.

6. Store finished slides in freezer.

D. Preparation of blank slides

Blanks should be made WEEKLY to check for contamination of reagents. Make slides following the above procedure but excluding an actual water sample. If there are many cells in a blank, filter or mix new reagents.

COUNTING PROCEDURE

EQUIPMENT:

Olympus BH2 microscope equipped with 100 watt Hg burner, oil immersion 100x S-plan objective, and reticle with Whipple grid Cargille type A immersion oil.

PROCEDURE:

Allow frozen slides to thaw before counting. Be sure slides are dry; condensed moisture can be removed by pressing slide between paper towels. Count 10 grids from each slide for a total of approximately 400 cells per slide (300 cells minimum). If bacteria are abundant, count a fraction of the grid. Randomize selection of fields by adjusting stage to next position with several turns while not looking at the slide.

CALCULATIONS:

To calculate cells per grid per ml (TGM):

Total cells counted/# grids counted/volume filtered (ml)

To calculate cells per liter:

TGM * tower-specific conversion factor * 1000

The tower-specific conversion factor is the number of grids in the stained area of a filter at a given magnification and can vary from one filter tower to another. Determine this number using stage micrometer to measure diameter of stained portion of several filters. Calculate area of stained portion and divide by area of grid to determine number of grids/filter.

To calculate bacterial biomass:

biomass (ug C/L) = (cells/L) * (8.2×10^{-9} ug C /cell)

The average volume of an UNDERC bacterial cell is 0.0215 μm^3. Assuming 0.38 g of C per cm^3 results in a conversion factor of 8.2 fg C per cell (Pace 1992).

REFERENCES:

Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescent microscopy. Applied and Environmental Microbiology 33: 1225-1228.

Pace, Michael L. 1992. Heterotrophic microbial processes. In: S.R. Carpenter and J.F. Kitchell, eds. The Trophic Cascade in Lake Ecosystems. Cambridge University Press. Cambridge, England.

(flowcyt97.doc)

FLOW CYTOMETRY

EQUIPMENT:

Glass scintillation vials
Plastic cone-shaped lids
0.2 um x 25 mm Millipore filters and Swinnex or 0.2 um x 25 mm Gelman Sterile astrodisks
Syringe (?10 cc)

REAGENTS:

Filtered (0.2 um) 50% aqueous photographic grade gluteraldehyde

PROCEDURE:

A. In the lab

Fill glass scintillation vial with 19 mL unfiltered sample and preserve with 1 mL filtered (0.2 um) 50% aqueous photographic grade gluteraldehyde. Send samples away for analysis.

This procedure has not been used since 1994.

(flagabun97.doc)

MEASUREMENT OF FLAGELLATE ABUNDANCE

EQUIPMENT:

0.8 µm x 25 mm Millipore filters (backing filters)
1.0 µm x 25 mm stained Nuclepore polycarbonate filters
Slides and cover slips
Filtration manifold
Filtration towers
Vacuum pump with tubing
10-100 µl pipettor
100-1000 µl pipettor
5-10 ml pipettor

REAGENTS:

0.033% proflavine solution
10% glutaraldehyde (100 ml 25% glutaraldehyde + 150 ml distilled water; keep refrigerated)
Cargille type A immersion oil
Distilled water

PROCEDURE:

A. Preperation of 0.033% proflavine solution

Add 33 mg proflavine to 100 ml distilled water and freeze in 10 ml aliquots in plastic scintillation vials.

B. Slide preparation

1. Prepare two filters for each sample. Place wetted backing filters and stained Nuclepore filters on filtration towers.

2. Gently stir sample and place 10 - 30 ml subsamples in filter towers.

3. Add 40.0 µl proflavine for each 10 ml of sample and stain for two minutes.

4. Add 1 ml cold 10% glutaraldehyde for each 10 ml of sample to make a 1% final solution; let sit for two minutes.

5. Filter under as low a vacuum as possible (<100 mm Hg).

6. Remove filters, allow to air dry, place on a labelled (lake, date, depth, volume filtered, filter tower used) slide between two drops of immersion oil and cover with a cover slip.

7. Store finished slides in freezer.

COUNTING PROCEDURE

EQUIPMENT:

Olympus BH2 microscope equipped with 100 watt Hg burner, oil immersion 60x S-plan APO objective (numerical aperture = 1.4), and reticle with Whipple grid Cargille type A immersion oil.

PROCEDURE:

Allow frozen slides to thaw before counting. Be sure slides are dry; condensed moisture can be removed by pressing slide between paper towels. Flagellates are counted using a 60x objective. Using the stage micrometer for measurements, count 5 mm or 10 mm "strips" of the sample. The width of a strip is the width of the grid. Count at least 50 flagellates per sample (40 minimum).

CALCULATIONS:

To calculate the number of flagellates per liter:

(totalF / #strips)*(tower cf / vol)*1000

Where:

totalF = total number of flagellates counted
#strips = the number of 10 mm strips counted
tower cf = tower-specific conversion factor
vol = volume of sample filtered (ml)

The tower-specific conversion factor is the number of 10 mm strips in the stained area of a filter at a given magnification and can vary from one filter tower to another.

REFERENCES:

Bloem, J., M.J. Bar-Gilissen, and T.E. Cappenberg. 1986. Fixation, counting, and manipulation of heterotrophic nanoflagellates. Applied and Environmental Microbiology 52: 2166-2172.

Haas, L.W. 1982. Improved epifluorescence microscopy for observing planktonic micro-organisms. Annals de L'Institut Oceanographique, Paris 58 (Supplement): 261-266.

Landry, M.R., L.W. Haas, and V.L. Fagerness. 1984. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. Marine Ecology Progress Series 16: 127-133.

(thymid97.doc)

MEASUREMENT OF BACTERIAL GROWTH – THYMIDINE INCORPORATION

EQUIPMENT:

15 ml disposable polypropylene test tubes with caps
0.2 µm x 47 mm Nuclepore polycarbonate filters
47 mm Gelman AE filters (backing filters)
Filtration manifold
Filtration towers
Vacuum pump with tubing
10-100 µl pipettor
100-1000 µl pipettor
5-10 ml pipettor
Incubators set at *in situ* temperatures

REAGENTS:

Methyl-^{3}H-Thymidine (Concentration: 1 mCi/ml, S.A. approx. 85 Ci/mmol, diluted to approximately 20µCi/100µl)
5% trichloroacetic acid (TCA) solution (500 g crystalline TCA + 1000 ml distilled water makes 50%; 100 ml 50% TCA + 900 ml distilled water to make 5%; refrigerate)
50% TCA
scintillation cocktail (Scintiverse BD)

PROCEDURE:

A. Preparation of ^{3}H-thymidine (Tdr) working stock and voucher

Dilute ^{3}H-Tdr to approximately 20µCi/100µl so that the final concentration of the sample is 23.0 nM. This is not sterile, so prepare only what you will use immediately. The dilution depends on the specific activity of the ^{3}H-Tdr, but approximately 2100 µl distilled water (0.2 µm filtered) + 500 µCi ^{3}H-Tdr makes enough for 24 samples at about 20 µCi per sample.

Check isotope stock by preparing two vouchers each time a new batch of working ^{3}H-Tdr stock is made: 50 µl working stock + 1950 µl (0.2 µm filtered) distilled water (makes 9.05µCi/2000µl solution). Place 100 µl of this solution in a labelled scintillation vial along with 10 ml of Scintiverse BD. Count the vouchers on the same scintillation counter that is used for counting samples.

B. Incubation procedure

1. Take 6 replicate 10 ml samples from the epilimnion (pool mixed layer) and place in clean, labelled (lake, date, depth, and live or control) polypropylene test tubes. Two of the six tubes should be control tubes; these should be killed immediately after adding the working stock (see below).

2. Add 100 µl (20 µCi) working stock ^{3}H-Tdr to each tube.

3. Immediately stop incorporation in two tubes with 2.0 ml of 50% TCA; these will serve as controls.

4. Incubate for one hour at *in situ* temperatures.

5. At end of incubation, stop "live" tubes with 2.0 ml of 50% TCA. (At this point, the samples may be placed in a refrigerator for several hours until it is convenient to filter them.)

6. Place thoroughly wetted backing (distilled water) and Nuclepore filters in filtration towers; filter contents of each tube (200 mm Hg vacuum pressure or less).

7. Rinse twice with ice cold 5% TCA (keep in ice bath) by adding 2 ml TCA to each tube and pouring onto filter.

8. Remove filter tower and carefully rinse edge of filter with 2 ml 5% TCA.

9. Fold filter, return it to test tube, cap, and freeze.

(DNAextract97.doc)

BACTERIAL PRODUCTION -- DNA EXTRACTION

EQUIPMENT:

100-1000 µl pipettor
5-10 ml pipettor
Refrigerated centrifuge
Centrifuge tubes
Ice bath
Vacuum pump with aspiration apparatus
Hot plate and water bath
Glass scintillation vials

REAGENTS:

Alkaline extractant
Ethylenediaminetetraacetic acid (EDTA)
Lauryl sulfate - sodium salt (SDS)
Sodium hydroxide
Carrier DNA solution
3 N HCl
50% trichloroacetic acid (TCA)
5% trichloroacetic acid (TCA)
Scintillation cocktail (Scintiverse BD)

PROCEDURE:

A. Preparation of alkaline extractant

Dissolve the following in 1L distilled water:
12 g sodium hydroxide
7.5 g EDTA
1 g SDS

B. Preparation of carrier DNA solution

Prepare a solution of 5 mg carrier DNA per ml of alkaline extractant. 10 ml is enough for 84 samples (50 mg DNA, 10 ml extractant).

C. Extraction

Add 5 ml alkaline extractant to each tube with filter; leave at room temperature for 15 minutes.

D. Precipitation

1. Remove filter with forceps and transfer extractant to centrifuge tubes in ice bath.

2. Turn on centrifuge and set temperature to 0°C.

3. To each of the centrifuge tubes, add:

0.5 ml 3 N HCl
1.0 ml 50% TCA
100 µl carrier DNA solution

4. Leave tubes in ice bath for 30 minutes; add ice as necessary to keep samples cold.

5. Centrifuge samples at 12000 rpm and 0°C for 15 minutes.

6. Aspirate supernatant, being careful not to aspirate any of the pellet. Add 3 ml 5% TCA.

7. Repeat steps 5 and 6 (centrifuge, aspirate, add TCA). Load tubes for the second spin so that the pellet is facing to the outside of the centrifuge.

E. Immerse tubes in 100°C water bath for 30 minutes; all of the pellet should dissolve. Cool to touch.

F. Centrifuge samples at 10000 rpm for 10 minutes at 10°C .

G. Place 1 ml supernatant in scintillation vial; add 10 ml scintillation cocktail; count in scintillation counter.

CALCULATIONS:

When calculating bacterial production from thymidine incorporation, we assume no dilution by unlabeled thymidine pools. It is possible to measure isotope dilution; see Moriarty (1986) for a discussion.

A. To calculate incorporation of ^{3}H-thymidine into DNA (TDRi, in pmol/L/h):

$$TDRi = \frac{(CPMs)\,(Vc)\,(TDRp)}{(Vs)\,(T)\,(Ceff)\,(DPMt)}$$

where:

CPMs = counts per minute for sample
Vc = volume correction; only 1 of 3 ml left at end of extraction is counted, so Vc = 3 (no units)
TDRp = amount of ^{3}H-Tdr added to each sample (pmol)
Vs = volume of sample used in incubation (L)
T = length of incubation (h)
Ceff = counting efficiency of fluor, determined from quench curve
DPMt = DPM for ^{3}H-Tdr added to sample

B. To calculate bacterial growth in cells per liter per hour:

cells/L/h = (TDRi) (cf)

where:

TDRi = ^{3}H-Tdr incorporated into DNA (pmol/L/h)
cf = a conversion factor: 5.2×10^{9} cells produced per nmol ^{3}H-Tdr incorporated (Smits and Riemann 1988).

C. To calculate bacterial production in ug C/L/h:

ug C/l/h = (cells/L/h) * (8.2×10^{-9} ug C/cell)

Determination of cell to biomass conversion factor: The average volume of an UNDERC bacterial cell is 0.0215 um^{3}. Assuming 0.38 g of C per cm^{3} (Lee and Fuhrman 1987, Simon and Azam 1989) results in a conversion factor of 8.2 fg C per cell.

REFERENCES:

Findlay, S.E.G., J.L. Meyer, and R.T. Edwards. 1984. Measuring bacterial production via rate of incorporation of ^{3}H thymidine into DNA. Journal of Microbiological Methods 2: 57-72.

Fuhrman, J.A. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Applied and Environmental Microbiology 39: 1085-1095.

Lee, S. and J.A. Fuhrman. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Applied and Environmental Microbiology 53: 1298-1303.

Moriarty, D.J.W. 1986. Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Advances in Microbial Ecology 9: 245-292.

Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series 51:201-213.

Smits, J.D. and B. Riemann. 1988. Calculation of cell production from [^{3}H] thymidine incorporation with freshwater bacteria. Applied and Environmental Microbiology 54: 2213-2219.

(leucine97.doc)

MEASUREMENT OF BACTERIAL PRODUCTION – LEUCINE INCORPORATION

This procedure has not been used since 1996.

EQUIPMENT:

Peristaltic pump with tubing
15 ml disposable test tubes or centrifuge tubes with screw caps
Incubators capable of maintaining in situ temperatures and heating to 85°C
Centrifuge tubes for extraction (minimum volume: 20 ml, caps should seal tightly) Filtration apparatus
Vacuum pump
0.45 µm x 25 mm Whatman cellulose nitrate filters
Scintillation vials

REAGENTS:

L-[4,5-^{3}H] Leucine (concentration: 1 mCi/ml; diluted to 15µCi/100µl)
50% trichloroacetic acid (TCA) solution
5% trichloroacetic acid (TCA) solution
80% ice-cold ethanol
Ethylene glycol monomethyl ether (EGME)
Scintillation cocktail

PROCEDURE:

A. Preparation of ^{3}H leucine solution and voucher

Dilute ^{3}H-Leu to 15µCi/ 100µl. This is not sterile, so prepare only what you will use immediately. 3.82 ml distilled water (0.2 µm filtered) + 670 µCi ^{3}H-Leu makes enough for about 42 samples.

Check isotope stock by preparing a voucher each time a new batch of working ^{3}H-Leu stock is made: 10 µl working stock + 990 µl (0.2 µm filtered) distilled water (makes 1.5µCi/1000ul solution). Place 100 µl of this solution in a labeled scintillation vial. Count the voucher on the same scintillation counter that is used for counting samples.

B. Sample collection

Using peristaltic pump, fill six centrifuge tubes at each depth. There should be no air bubbles evident. Store in cooler filled with water from sample depth. Using 8 liter nalgene bucket, thoroughly drench Limno team.

C. Leucine addition

1. Carefully uncap tubes and add 100 µl ^{3}H-leucine solution to each tube. Note time. Replace caps on 5 of the six tubes, the remaining tube will serve as a control.

2. Pour the control sample into one of the extraction tubes; immediately add 2 ml 50% TCA and cap.

3. Incubate each set of tubes at in situ temperature for 45 minutes.

4. While samples are incubating, prepare the rest of the extraction tubes by placing 2 ml 50% TCA in each tube.

5. Transfer samples to extraction tubes and cap. Heat in 75°C water bath for 30 minutes, then allow to cool completely.

6. Filter contents of each tube through wetted (distilled water) Whatman filter.

7. Rinse extraction tube with 2 ml 5% TCA and pour through filter.

8. Remove filter towers and rinse each filter twice with 0.5 ml 5% TCA, once with 0.5 ml 80% ice-cold ethanol, and once with 0.5 ml distilled water. Filter until very dry.

9. Place each filter in a labeled scintillation vial. Add 1 ml EGME to dissolve filter. Allow to dissolve for at least 24 hrs.

10. When filter has completely dissolved, add 10 ml scintillation cocktail, allow to dissolve for at least 48 hrs, and count on scintillation counter.

CALCULATIONS:

When calculating bacterial production from leucine incorporation, we assume no dilution by unlabeled leucine pools. It is possible to measure isotope dilution; see Simon and Azam (1989) or Chróst (1990) for a discussion. All calculations (except part D) from Simon and Azam, 1989.

A. To calculate ^{3}H-leucine incorporation (LEUi, in moles/L/h):

$$LEUi = \frac{(CPMs)\,(LEU)}{(Ceff)\,(DPMt)\,(Vs)\,(T)}$$

where:
CPMs = counts per minute for sample
LEU = amount of ^{3}H-leucine added (mol)
Ceff = counting efficiency for fluor, calculated from quench curve
DPMt = DPM for ^{3}H-leucine added to sample
Vs = sample volume (L)
T = length of incubation (h)

B. To calculate bacterial protein production (BPP, in g/L/h) from ^{3}H-leucine incorporation:

BPP = (LEUi) (100/7.3) (131.2)

where:
LEUi = rate of leucine incorporation (mol/L/h)
100/7.3 = 100/mol% of leucine in protein
131.2 = the formula weight of leucine

C. To calculate bacterial carbon production (BCP, in g/L/h) from BPP:

BCP = (BPP) (0.86)

D. To calculate bacterial production (BPRO in cells/L/h) from bacterial carbon production:

$$BPRO = \frac{BCP}{(\text{cell carbon content})}$$

where:
BCP = bacterial carbon production (g/L/h)
cell carbon content = 8.2 fg C per cell

The average volume of an UNDERC bacterial cell is 0.0215 µm^{3}. Assuming 0.38 g of C per cm^{3}, cell carbon content is 8.2 fg C per cell (Pace 1992).

REFERENCES:

Chróst, Ryszard J. 1990. Application of the isotope dilution principle to the determination of substrate incorporation by aquatic bacteria. Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie 34: 111-117.

Kirchman, D.L., E. K'nees, R. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Applied and Environmental Microbiology 49: 599-607.

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Pace, Michael L. 1992. Heterotrophic microbial processes. In: S.R. Carpenter and J.F. Kitchell, eds. The Trophic Cascade in Lake Ecosystems. Cambridge University Press. Cambridge, England.

Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series 51: 201-213.

(leumicro97.doc)

MEASUREMENT OF BACTERIAL PRODUCTION- LEUCINE INCORPORATION USING MICROCENTRIFUGATION METHOD

EQUIPMENT:

Disposable plastic cups and lids
Soup thermoses
60 mL BOD bottles
Styrofoam thermoses
Peristaltic pump
Cooler
2 mL Eppendorf disposable microcentrifuge tubes and caps
Rubber septum caps
Microcentrifuge
Vortex mixer
Pump with aspirating tube and pipette
Syringe (60 cc) + needle
Syringe (~3 cc) + needle
500-1500 uL pipettor
100 uL pipettor ("hot")
Incubators set at *in situ* temperatures
Rubber gloves

REAGENTS:

L-[4,5-^{3}H] Leucine (Concentration: 1 mCi/mL , S.A. :approx. 60 Ci/mmol)
5% trichloroacetic acid (TCA) solution (500 g crystalline TCA + 1000 ml distilled water makes 50%; 100 ml 50% TCA + 900 ml distilled water to make 5%; refrigerate)
50% TCA
Scintillation cocktail (Scintiverse BD)

PROCEDURE:

A. Preparation of ^{3}H leucine solution and voucher

^{3}H leucine solution is prepared weekly. Dilute stock isotope to 1.4 µCi/100 µL, such that the final concentration of the sample is 17.5 nM. This is what the Cascade protocol has used. Smith and Azam have noted using a 20 nM concentration in their methods paper. The dilution depends on the specific activity of the ^{3}H leucine solution, but approximately 115 µL of ^{3}H leucine should be added to 8085 µL of distilled water (0.2 um filtered) to make enough for about 80 samples and two vouchers.

Check isotope concentration by preparing two vouchers for each new batch of working solution made: 50 µL working stock + 1950 µL (0.2 um filtered) distilled water. Place 100 uL of this solution into each of two labeled scintillation vials. Add at least 10 mL of scintillation cocktail and measure on the LSC.

B. Sample collection

Because human skin has leucine on it, it is easy to contaminate water samples. EXTREME CARE SHOULD BE EXERCISED TO AVOID BOTH DIRECT AND INDIRECT CONTACT OF SKIN WITH ANYTHING THAT TOUCHES THE SAMPLES.

The depths are as follows:

<u>Oxic depths (1-4):</u>
Paul Lake - 1, 2, 3, 4 m
Peter Lake - 1, 2, 4, 5 m
West Long Lake - 1, 2, 3, 4 m
East Long Lake - 0.5, 1, 1.5, 2 m

<u>Anoxic depths (5-6):</u>
Paul - 5, 9 m
Peter - 7, 9 m
West Long Lake - 6, 9 m
East Long Lake - 4, 9 m

The oxic samples are pumped into four disposable plastic sample cups, each in its own soup thermos. The pump should first be rinsed by allowing 1 L of water from the appropriate depth to pass through the tubing. The plastic cup is then rinsed three times, filled, and placed into its soup thermos, which is filled with water from the same depth.

Water from the two anoxic depths is pumped into 60 mL BOD bottles. Three replicates are taken from each depth for a total of six bottles. Again, the pump should first be rinsed by allowing 1 L of water from the appropriate depth to pass through the tubing. The bottles are rinsed three times, filled to overflowing, then capped in such a way that no air bubbles remain inside the bottle. They are then placed into two insulating (styrofoam) containers, each of which are filled with water from the appropriate depth.

Both the soup thermoses and the styrofoam containers should be placed into a cooler until it is time for the incubation procedure.

C. Leucine incubation

Remember that human skin has leucine on it, so it is easy to contaminate water samples. For this reason, gloves should be worn at all times. It is important not only to wear gloves, but to also be sure that these gloves do not come into contact with skin before they are used. Anything that touches the samples must not be touched, either directly or indirectly, by skin.

Twenty microcentrifuge tubes are used per day. Three replicate tubes are created for each of six depths. In addition, two "kill" controls are created using water from Depth 1 and Depth 6.

1. Oxic Depths (1-4)

Pipet 1.5 mL sample volumes into each tube. Add 100 uL of isotope solution and immediately stop bacterial production in the (Depth 1) “kill” with 0.3 mL of 50% TCA. Incubate samples for 45 minutes at *in situ* temperature, then stop bacterial production in the “live” samples with 0.3 mL of 50% TCA.

2. Anoxic Depths (5-6)

Extreme care should be taken to avoid contact of sample with air.

Add 100 uL of isotope solution to tubes. Place rubber septums on top of tubes. Remove air from tubes with a 60 cc syringe and needle, creating a vacuum. Using a 5 mL syringe, add approximately 1.5 mL of sample to tubes (try to place needle into hole created by previous puncturing of septum.) The vacuum should cause the sample to leave the syringe. After adding sample to (Depth 6) “kill,” immediately stop bacterial production by removing septum and adding 0.3 mL of 50% TCA. Allow samples to incubate at *in situ* temperature for 45 minutes, then add 0.3 mL of 50% TCA to “live” samples as well.

D. Centrifugation

1. Turn on the microcentrifuge with the switch on the back of the machine near the electrical cord input. Open the microcentrifuge lid with the black lever on the right side. This only works when the microcentrifuge is on. To open the cover when the machine is off, use the metal rod and insert in the hole at the front (upper right) of the centrifuge.

2. Remove the clear plastic rotor cover. Make sure the rotor is on tight using the silver hex wrench. This wrench fits on the nut in the center and tightens clockwise. Load samples into the openings making sure the rotor is balanced with samples and replace the clear plastic cover. Shut the microcentrifuge lid.

3. The centrifuge has a timer which can automatically be set using the rotating wheel on the front of the machine. Check to see that it is set for 10 minutes. The speed dial should be set at 14000 rpm. The digital readout displays the speed of the rotor while running. Adjust if not stable at 14000 rpm.

4. Start the microcentrifuge by pushing the start/stop lever down towards "start". The machine will not run if the lid is not properly closed. Also, the lid cannot be opened while the rotor is spinning. The centrifuge will stop when it is done with its timed cycle.

5. When the rotor stops spinning and the light stops flashing and/or you hear a click, the lid can be opened with the black lever to the right. Remove the clear plastic rotor cover and remove samples.

E. Sample handling

1. After all samples have been "killed", centrifuge for 10 minutes at 14000 rpm.

2. Aspirate the liquid from the vial, taking care to remove **all** liquid.

3. Add 1.5 mL 5% TCA to each tube and vortex for 2-5 seconds.

4. Centrifuge again for 10 minutes at 14000 rpm, and aspirate liquid.

5. Add 1.5 mL scintillation cocktail (Scintiverse BD).

6. Vortex tubes, place in glass scintillation vial, and count in scintillation counter.

(commresp97.doc)

MEASUREMENT OF COMMUNITY RESPIRATION

EQUIPMENT:

4 clear glass 300 ml BOD bottles
4 "dark" 300 ml BOD bottles (cover bottles with an opaque material such as foil or electrical tape)
Peristaltic pump and tubing
Incubation lines (constructed so bottles will hang at a depth of one meter)
1-5 ml pipet
Balance
400 ml glass beaker
Stir plate and stir bar
10 ml buret with 0.05 ml subdivisions
Aluminum foil

REAGENTS:

$MnSO_4$ solution
Alkaline iodide sodium azide reagent
H_2SO_4 (concentrated)
Starch indicator solution
0.025N Sodium thiosulfate solution (filtered)

PROCEDURE:

A. Sample collection and incubation

1. At each lake, fill eight BOD bottles (4 light, 4 dark) with water pumped from 1 m. To fill, insert tubing to the bottom of the bottle, overfill once completely, and slowly remove tubing. Be careful not to introduce any air bubbles.

2. Stopper the four dark bottles, cover top of each bottle with foil to exclude all light, attach the incubation line and place them in the lake. Note time.

3. Immediately fix the remaining bottles by adding 2 ml $MnSO_4$, then 2 ml alkaline iodide sodium azide. Stopper bottles and shake vigorously.

4. Shake all bottles vigorously again before returning to lab (approximately 30 minutes after initial shaking.)

5. Return to the lab and titrate.

6. Retrieve the dark bottles 24 hours later. Fix as above, note time, return to lab and titrate.

B. Titration

Note: Sodium thiosulfate solution contains a bactericide which forms crystals that can clog a buret. To remove crystals, filter only as much of the solution that you will need using a 47 mm GFF filter. Discard any unused, filtered sodium thiosulfate.

1. Add 2 ml concentrated H_2SO_4, re-stopper bottle, and shake until the precipitate has completely dissolved.

2. Tare a glass beaker on the balance. Pour 100 ml of sample into the beaker and record weight.

3. Place stir bar in beaker and titrate with 10 ml buret containing Na thiosulfate solution. Add titrant until pale yellow (straw) color is present, add two drops starch indicator solution to get uniform blue color, and carefully but continuously titrate to a colorless end-point. Blue color will return after 15-20 seconds; this can be ignored. Record volume of titrant used. If replicates differ by more than 0.05 ml, perform titrations again.

CALCULATIONS:

Community respiration is calculated by subtracting the average of the dark bottle O_2 values from the average of the light bottle O_2 values.

To calculate mgO2/L in a sample:

$$\frac{(\text{ml titrant})(\text{N thiosulfate})(8000\text{mg/mol})}{(\text{ml titrated})\left(\frac{\text{ml BOD bottle} - 4}{\text{ml BOD bottle}}\right)}$$

(ml titrated) is determined by weighing the sample before titration and assuming a density of 1.

(Nathio.doc)

SODIUM THIOSULFATE STANDARDIZATION

EQUIPMENT: 10 ml buret with 0.05 ml subdivisions
400 ml glass beaker
stir plate and magnetic stir bar

REAGENTS: potassium iodide
H_2SO_4 (concentrated)
0.02N $KH(IO_3)_2$
distilled water
0.025N sodium thiosulfate solution

PROCEDURE:

1. Dissolve 2g KI in 150 ml distilled water.
2. Add 1-2 ml concentrated H_2SO_4.
3. Add 20 ml 0.02N $KH(IO_3)_2$
4. Dilute up to 200 ml with distilled water.
5. Titrate entire 200 ml in 4 equal portions.

CALCULATIONS:

$N1*V1 = N2*V2$ $N2 = (N1*V1)/V2$

where N1 = normality of KH(IO3)2 (=0.02)
V1 = volume KH(IO3)2 (=5 ml)
N2 = normality of Na thiosulfate
V2 = volume Na thiosulfate titrated (ml)

REFERENCES:

Strickland, J.D.H., and T.R. Parsons. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada.

Wetzel, R.G., and G.E. Likens. 1991. Limnological Analyses. Second edition. Springer-Verlag. New York.

(cellvol97.doc)

This procedure has not been used since 1994.

MEASUREMENT OF BACTERIAL CELL VOLUME

PHOTOGRAPHING BACTERIA FOR SIZE MEASUREMENTS

EQUIPMENT:

Olympus BH2 microscope equipped with 100 watt Hg burner, oil immersion 100x S-plan objective, and 35mm camera with shutter release (place a 5x magnifier between the microscope and the camera)
Cargille type A immersion oil
TMAX 400 black and white film
Micrometer slide marked in 10 µm increments or less

PROCEDURE:

1) Allow frozen slides to thaw before photographing. Be sure slides are dry; condensed moisture can be removed by pressing slide between paper towels.

2) Photograph (use these camera settings -- ASA: 1600, exposure: +2) a micrometer slide between each group of slides to keep slides from different samples separate. Slides of the micrometer will also be used for image analysis system calibration later.

3) Photograph enough fields from a slide so there will be at least 100 measurable cells. The exposure time is quite long (20-30 seconds), so take care not to disturb the microscope. Randomize selection of fields by adjusting stage to next position with several turns while not looking at the slide.

FILM DEVELOPMENT

EQUIPMENT:

Developing tank
Scissors
Can opener
D-76 developer
Stop solution
Ffix solution

PROCEDURE:

1) In complete darkness, use the can opener to pry one end of the film case off, unroll film, and cut as close to the spool as possible.

2) Load film in developing tank.

3) Develop with D-76 for 21-22 minutes (or use TMAX developer and develop for 8-10 minutes), agitating at 30 second intervals. Pour out developer, rinse once with tap water (rinse is optional).

4) Stop in stop solution for 30 seconds with constant agitation. Pour out stop solution, rinse once with tap water (rinse is optional).

5) Fix 4-5 minutes in fixer with constant agitation.

6) Rinse film for 15 minutes with running water. Add Photo-flo for 30 seconds. Remove excess water with squeegee and hang in drying cabinet.

7) Assemble and label slides.

IMAGE ANALYSIS

All of our image analysis is done with the Olympus Cue-2 image analysis system; instructions for its use can be found in the manual.

Calibration: Measure the distance between two of the subdivisions on one of the slides of the micrometer several times and use the average of those measurements to calculate a conversion factor that will convert the unitless measurements obtained with the image analysis system to microns.

CALCULATIONS:

We use one equation to calculate cell volume, regardless of cell shape. The two measurements needed for the calculation are area and perimeter. These measurements should be converted to microns before performing the calculations. To calculate cell volume:

$$\text{VOLUME} = 8.5\ (\text{AREA}^{2.5})\ (\text{PERIMETER}^{-2})$$

REFERENCES:

Bjornsen, P.K. 1986. Automatic determination of bacterioplankton biomass by image analysis. Applied and Environmental Microbiology 51: 1199-1204.

(cilabun97.doc)

This procedure has not been used since 1994.

CILIATE ABUNDANCE

SAMPLE COLLECTION:

Pour 100 ml unfiltered lake water into jar containing 1 ml Lugol's solution.

Lugols solution: Dissolve 10 g I_2 and 20 g KI in 180 ml distilled water and 20 ml glacial acetic acid.

COUNTING PROCEDURE

EQUIPMENT:

Inverted microscope
Graduated cylinders (100 ml)
10 ml plankton settling chambers
aspiration apparatus

PROCEDURE:

1) Pour entire sample into a 100 ml graduated cylinder; rinse sample jar into cylinder with distilled water. Let sample settle overnight.

2) Aspirate all but approximately 8 ml of sample, being careful not to disturb settled sample. Pour sample into settling chamber, rinse cylinder with 2 ml distilled water and pour into settling chamber. Allow sample to settle for several hours or overnight.

3) Count ciliates on inverted microscope by scanning the entire chamber at 150x.

REFERENCES:

Pace, M.L., and J.D. Orcutt, Jr. 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography 26: 822-830.

This procedure has not been used since 1994.

MICROZOOPLANKTON ABUNDANCE:

SAMPLE COLLECTION:

Filter 1 liter of lake water through a 35 um seive. Do this with the mesh submerged in water so that the organisms are not poured directly against the mesh and subsequently deformed. Add approximately 3 oz soda water to narcotize the plankton, let sit a few seconds and drain slowly. Pour off into a sample jar, containing 40 ml of sucrose-formalin solution. Sample jars should be weighed prior to the addition of preservative or sample so that the proportion of sample counted can be determined later on.

Record the weight on the label of the sample jar. Rinse net thoroughly into sample jar with DI.

Sucrose-Formalin Solution: 60g sucrose, 100 ml 37% formalin, 825 ml DI. Mix thoroughly.

COUNTING PROCEDURE:

Microzooplankton are counted using 10 cc settling chambers and the Olympus inverted microscope back at IES. To remove the formalin and concentrate the sample, pour it through a 35 um mesh cup. Rinse the contents of the cup back into the sample jar using distilled water. Weigh the sample and jar, record weight on counting sheet. Mix the sample by drawing up 10 ml into an Oxford pipet several times. Remove 10 ml of sample and place in settling chamber. Allow sample to sit for a minute before placing cover on chamber. The sample should be allowed to settle completely before counting (a couple hours). Count the contents of the entire chamber. At least 100 of the most abundant rotifers should be counted.

To calculate abundance when only a portion of the sample was counted: The weight of concentrated sample is the weight of the concentrated sample and the jar (g) - empty jar weight (g) (should be written on the label). This is the volume (in ml) if you assume sample density = 1. From that, calculate the fraction of sample counted and abundance of each species (number per liter).

(pH97.doc)

pH MEASUREMENT

EQUIPMENT:

300 mL BOD bottle
pH meter

REAGENTS:

pH standards (10, 7, 4)

PROCEDURE:

pH

A. IN THE FIELD:

After three rinses, fill a 300 mL BOD bottle by "glugging" bottle just below the surface of the water.

B. IN THE LAB:

1) Setup and calibrate pH meter using 2 standards that bracket the pH of the water being measured. This is done as follows:

a) Make sure pH electrode is filled at least one inch above the reference junction with Ag/AgCl filling solution. Turn the instrument on and wait until it starts measuring.

b) Remove probe from soak bottle

c) Rinse with milli-q

d) Uncover the hole at the upper end of the probe

e) Press 2nd, then CAL

f) When "C1" appears, put the probe in the lower pH buffer (either 4 or 7) and wait for it to flash at the appropriate pH. Press YES.

g) Remove the probe and rinse thoroughly.

h) When "C2" appears, put the probe in the higher pH buffer (either 7 or 10) and wait for it to flash at that pH. Press YES.

i) Check to be sure that the calibration curve has a slope that is close to 1.00.

j) Rinse probe and start measuring.

3) To measure:

a) Place probe in enough sample water to cover it for 15 minutes, discard sample, repeat.

b) Place probe in enough sample water to cover it for 5 minutes, then read pH (reading should be stable for 1 minute).

4) Upon completion, rinse the probe, turn off meter and fit probe in soak bottle, making sure the measuring end of the probe is submersed in pH 7 buffered KCL storage solution. Buffer solutions should be replaced weekly.

(dic97.doc)

This procedure has not been used since 1995.

DIC

EQUIPMENT:

Cooler
Peristaltic pump and tubing
BOD bottle
60 mL syringe

REAGENTS:

0.2 N sulfuric acid
CO2/He gas standard canister

A. IN THE FIELD:

1) Partially fill a cooler with surface water from the lake.

2) Using the peristaltic pump, fill 2 BOD bottles with lake water drawn from 0.2 m (one bottle for DIC and one for pH). To fill, insert tubing to the bottom of the bottle, overfill once completely, and slowly remove tubing. Cap the bottle, taking care not the trap any air in the bottle. Immediately place the bottle in the cooler.

B. IN THE LAB:

3) Draw 25 ml of water from the BOD bottle into a 60 ml syringe, taking care not to introduce any air.

4) Add 0.2 ml of 0.2 N Sulfric Acid to the syringe.

5) Draw 25 ml of He into the syringe.

6) Shake syringe vigorously 100x

7) Inject 10-15 ml of the gas into the GC, taking care not to inject any water.

8) Repeat steps 3-7. If bad replication repeat steps 3-7 until samples replicate.

(pocdoc97.doc)

PARTICULATE AND DISSOLVED ORGANIC CARBON/COLOR

EQUIPMENT:

25 mm GF/F filters (ashed)
Petri dishes
Filter tower apparatus and pump
Glass scintillation vials
Plastic cone-shaped lined caps
60 mL HDPE bottles
spectrophotometer

REAGENTS:

2N H_2SO_4
Distilled water

A: To ash filters:

1. Place filters in a foil boat and cover. Put in 450^{o}C oven for 2 hours.

B: POC / PON

1. Pour 100-300 ml duplicate samples from each depth through 153 um mesh to remove large zooplankton.

2. Filter samples through ashed GF/F filters at less than 200 mm Hg pressure. Remove filters from towers, fold in half, and place two replicates in one labeled petri dish. Be sure to indicate mL of water filtered when labeling. Place dish in drying oven.

3. After filters have dried (a couple of days), remove dish from drying oven and store in desiccator. Analyze samples at IES.

C: DOC

1. Pipet 20 mL of filtrate (from POC procedure) into labeled glass scintillation vial and acidify with 200 uL 2N H_2SO_4. Analyze samples at IES.

D. COLOR

1. Fill 60 mL HDPE bottle with filtrate (from POC procedure). Only one replicate from each sample is needed. Store in refrigerator until it is convenient to analyze samples on a spectrophotometer.

2. Set spectrophotometer to 440 nm. After calibrating with distilled water, rinse cuvette with 10 mL of filtrate. Remove rinse, then fill with 30 mL of filtrate and measure absorbance. Continue in this manner until all samples have been measured.

2. To estimate the amount of machine drift, measure the absorbance of distilled water after measuring sample

(isotope97.doc)

C_{13}/C_{12} ISOTOPE ANALYSIS

EQUIPMENT:

25 mm GF/F filters (ashed)
Petri dishes
Filter tower apparatus and pump

A. Ash filters at 450^{o}C oven for 2 hours (as for POC/DOC procedure).

B. 1. Pour duplicate samples from epilimnetic water through 153 um mesh to remove large zooplankton.

2. Filter samples through ashed GF/F filters at less than 200 mm Hg pressure. Filter enough water to thoroughly color the filters. Remove filters from towers, fold in half, and place on top of large GF/F filters in labeled petri dish. Be sure to include mL of water filtered when labeling. Place dish in drying oven.

3. After filters have dried (a couple of days), remove dish from drying oven and store in desiccator. Have samples analyzed upon return to IES.

(DielO297.doc)

DIEL O_2 MEASUREMENT

EQUIPMENT:

YSI Model 6000 Environmental Monitoring System (Sonde)
PC 6000 Software
Floating platform

PROCEDURE:

A. Weekly checks and calibration:

1. Check the oxygen sensor for bubbles in the KCL solution, the integrity of the teflon membrane, and for rust spots on the electrode. Add KCL solution and change the membrane and/or sand away rust spots if necessary.

2. Refresh batteries with 8 rechargeable or disposable C-cell alkaline batteries. Apply O-ring lubricant to the O-ring inside the battery chamber lid if necessary.

3. Calibrate the oxygen sensor by inserting the sonde into a calibration cup with a wet sponge inside. Once in PC6000 software, select RUN from the sonde main menu and DISCRETE SAMPLE from the RUN menu. Set sample interval to 4 seconds. After 10-15 minutes, select CALIBRATE from the sonde main menu. Select DO% and enter current barometric pressure in atmospheres. Calibration is now in progress. As an extra calibration check, 4 BOD bottles are filled with lake water at a depth of 1m at a recorded time when the sonde is taking that lake's epilimnetic readings. The BOD bottles are fixed, and titrated back at the lab. Oxygen concentrations are compared between methods.

B. Field Sampling:

1. Program the sonde in unattended sampling mode to take readings at 5-minute intervals for desired time. Program some extra time in so that the sonde is logging measurements when put in the water.

2. When programming is completed (indicated by message: *INSTRUMENT IS IN UNATTENDED MODE*), replace the PC interface cable with the waterproof cap. Apply the enclosed lubricant to screw threads for a tight seal if necessary.

3. Place the sonde in the desired lakes for the desired period of time. Hang the sonde from floating platforms on each lake (same platforms used for the anemometer/data logger). Record starting and ending times at each depth in each lake. After the 4 day sampling period, bring the sonde back to the lab, clean off all surfaces, and download data. Data are downloaded as .dat files and need to be exported from PC6000 program. They then can be imported into a spreadsheet as ASCII files. These files first appear in the spreadsheet as if each row was only in the first column. Data can be separated into columns by parsing the ASCII file. Page breaks must first be deleted. The @value (cells) command can be used to convert parsed cells (text) into values.

4. If the computer is not able to communicate with the sonde, it has been suggested by YSI to completely remove the battery cover for 10-15 seconds and then retry communicating.

This procedure has not been used since 1996.

WIND MEASUREMENT

EQUIPMENT:

Anemometer (Young Co., Model 05103)
Campbell data logger
Floating platforms
XTALK downloading program

PROCEDURE:

A. Programming the data logger

1. Wiring from the anemometer to the data logger:

Channel
1 H Green
Pulse inputs
3 Red
3 G Black
Excitation
1 White (Blue)

2. Programming

Each time the datalogger is turned off and on, it must be reprogrammed to log data. The following programming procedure first lists a memory location number followed by the intended program code. The correct series of location numbers should appear when selecting ENTER after each inputted program code.

PROGRAM:

			COMMENTS
	*1 A		
	01:	2	2 second execution interval
	01:	P04	
	01:	01	
	02:	05	
Wind	03:	01	
Direction	04:	01	
	05:	0000	
	06:	1000	
	07:	0001	

	08:	.355	to enter a decimal, key in D
	09:	0	
	02:	P03	
Wind Speed	01:	01	
	02:	03	
	03:	01	
Wind Speed	04:	0002	
	05:	.0490	
	06:	0	
	03:	P10	battery voltage
	01:	0003	loc.
	04:	P89	If
	01:	0001	loc. (wind direction)
	02:	04	is less than
	03:	0	0
	04:	30	then do
	05:	P30	z=f
	01:	0	f=0
	02:	0001	z=loc. (wind direction)
	06:	P95	end
	07:	P92	If time is
	01:	0	0 minutes into a
	02:	0060	60 minute interval
	03:	10	set output flag
	08:	P77	store real time
	01:	0110	julian day + hour-min
Don't type this 09:		P76	wind-vector
in if don't want	01:	01	reps.
wind direction	02:	0	sensor type (polar) saved.
	03:	0002	wind speed input loc.
	04:	0001	wind direction input loc.
	10:	P71	average
	01:	01	reps.
	02:	0002	loc. (wind speed)
	11:	P74	minimize
	01:	01	reps.

02:	10	store time (hour-min)
03:	0002	loc. (wind speed)
12:	P73	maximize
01:	01	reps.
02:	10	store time (hour-min)
03:	0002	loc. (wind speed)
13:	P52	standard deviation
01:	01	rep
02:	0002	loc. (wind speed)
03:	0004	store in location 4
04:	2000.0	no. of samples included in SD
14:	P70	sample
01:	01	rep
02:	0004	loc. (SD of wind speed)
15:	P70	sample
01:	01	rep
02:	0003	loc. (battery voltage)

Set date and time
*5 A
:HH:MM:SS
A 05: xx (Year)
A 05: xxxx (Julian day)
A 05: HH:MM (Hour:Minute)
(note whether it's Daylight Savings Time or Standard Time - subtract an hour from DST to get ST)

*0 log data
*6 A... scans current readings-should be 3:
01: wind direction (degrees)
02: wind speed (m/s)
03: battery voltage
(B scans backwards, A goes in advance)

*7 A scans previous hour's final storage data

B. Field data collection

After the data logger is programmed and loading information, the anemometer/data logger can be placed in the field. Field location and sampling time should correspond with the oxygen sensor (sonde), such that it is on Peter Lake for two days and West Long Lake for two days. Attach the anemometer/data logger to the floating platform near the

center of the lake (also used for the sonde). Connect the anemometer and data logger via the connector plug. Record times when it is put on and taken off of the lakes.

C. Downloading data

1. The datalogger stores each data point in a memory location number. Every hour, 14 data points are stored, as follows:

    1. Identifier (a code, should all be the same)
    2. Julian day
    3. Hour - minute
    4. Ave. wind speed (m/s)
    5. Ave. wind vector magnitude (m/s)
    6. Ave. wind vector direction (degrees)
    7. Std. dev. of vector direction
    8. Ave. wind speed (m/s)
    9. Minimum wind speed (m/s)
    10. Time of min (hour - min)
    11. Maximum wind speed (m/s)
    12. Time of max (hour - min)
    13. Std. dev. of wind speed
    14. Battery voltage

After 1 day, the location number should be 337 (14x24 hrs.). After using up all of its location numbers, the datalogger will start rewriting at location #1, writing over what was previously stored in location #1. To find out the number of locations available, key in *A 01:... 02:... 03:final storage locations 04:...Don't type any numbers in here or you will lose data. To avoid reprogramming, let the datalogger log data continuously. In order to retrieve that portion of data when the datalogger was connected to the anemometer on the lakes, note the location number just before each week's deployment and after retrieval. To dump the relevant data, connect the datalogger and computer and call up XTALK software. Set baud rate on computer and datalogger to 300. To check datalogger, key in *4 A. 02: should be baud rate (00=300, 1=1200, 02=9600 ...). Set port 2 on computer and create a filename. When the computer is ready (ESC FOR ATTENTION should be at bottom of screen and the cursor at the top), key in *9 A. At 01: prompt, key in start location number (recorded before week's sampling) and then A. At 02: prompt, key in end location number (after week's sampling) and A. At 03: prompt, key in any number and A. Data should now be downloading. After the end location number is reached, escape and quit the program. Data is saved under given filename as a text file. Enter into IFT21 software to delete extraneous addition signs. You must rename the file in this software.

(ysi97.doc)

YSI D.O. METER CALIBRATION

PROCEDURE:

Refill the probe with KCL solution and change the membrane if necessary. Turn on the instrument and leave on for at least 5 minutes. Make sure salinity is set to FRESH. Switch to ZERO and adjust O_2 ZERO control until display reads 00.0. In the lab where temperature is constant, place the probe in its storage bottle with a wet piece of paper towel. Switch control to % and allow to sit for several minutes. When the reading has stabilized, unlock and adjust O_2 CALIB control to 100% and relock. The instrument is now calibrated.

(apaproc97.doc)

ALKALINE PHOSPHATASE ACTIVITY

Note: This method last used during the summer of 1995.

EQUIPMENT:

Turner 450 Fluorometer fitted with:
1. Quartz-halogen lamp
2. Emission filter -NB460
3. Excitation filter -NB360

12 x 75 mm disposable glass culture cuvettes (should be boiled in distilled water for at least 20 min., dried and cooled before each use)
1-5 mL Oxford pipettor
100-1000 µL automatic pipettor
Stopwatch/timer
Incubator set at the surface temperature of the lake

NOTE
-**Change filters with fluorometer off!** (Remember that chlorophyll analysis filters are different from APA analysis filters.)
-**Make sure Fluorometer has been calibrated for APA** (see **Fluorometer Calibration for APA**).

REAGENTS:

165 µM 4-methyllumbelliferyl-phosphate (MUP: m.w. = 256.2) in .1 M Tris buffer, frozen in aliquots
(see **Preparation of MUP for APA**)

OVERVIEW:

The procedure involves putting an excess amount of a phosphate-containing organic complex into a cuvette. The phosphate is cleaved from the organic complex by the phosphatase enzyme in the lake sample, and the organic complex that has had phosphate removed is measured fluorometrically. The samples are run for standard time spans at a constant temperature. End-product formation is compared against blanks that have been sterilized to denature the enzymes.

PROCEDURE:

****NOTE**The Span knob on the fluorometer must be taped in place. If it is moved, the fluorometer must be recalibrated.** Calibration of the fluorometer is typically performed at the beginning of the field season, or when a bulb is changed.

1. Insert the proper filters into the fluorometer (NB460 Emission and NB360 Excitation). Turn on fluorometer and allow to warm up for at least 1 hour.

2. Use about 500 mL of depth 6 lake water as a blank. This blank is to be boiled for at least 5 minutes. Cool the blank water to room temperature.

3. Dispense 3.0 mL of each sample into cuvettes, making 3 replicates of each sample depth and 3 replicates of the blank. Allow samples to equilibrate to ambient lake temperature in the incubator (about 10 min.).

4. Slowly thaw the frozen MUP substrate. MUP should finish thawing just as samples are finished equilibrating. Quickly add 300 µL of substrate to each cuvette and mix by thumping the bottom of the cuvette several times. The final substrate concentration is 15 µM (saturating).

5. Read and record the fluorescence of each sample in an established order as quickly and consistently as possible on the gain for which the fluorometer is calibrated (see **Calibration of Fluorometer for APA**). Start the timer as the first cuvette is placed in the fluorometer (t=0). Place the cuvettes in the incubator.

6. Remove cuvettes from incubator and repeat step 5. at 10 min intervals (record exact interval). Do 4 intervals. (To 40 minutes on timer.)

CALCULATIONS:

Alkaline phosphatase activity (**APA**) (not normalized to algal biomass) is calculated as:

$$\mathbf{APA = m * ((\Delta F_S - \Delta F_B)/\Delta T)}$$

where $\mathbf{\Delta F_S}$ is the mean change in fluorescence of the sample over the several sampling intervals ($\mathbf{\Delta F_S}$ for the intervals should be constant since the reaction should proceed with linear kinetics) and $\mathbf{\Delta F_B}$ is the change in fluorescence of the blank (usually zero). $\mathbf{\Delta T}$ is the length of the monitoring interval (usually 10 minutes), and **m** is the calibration factor (door factor) relating fluorescence units to end-product (MUF) concentration (see **Fluorometer Calibration for APA**).

REFERENCES:

Pettersson, K. 1980. Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. Arch. Hydrobiol. 89:54-87.

(mup97.doc)

PREPARATION OF MUP FOR APA

EQUIPMENT:

autoclave
pH meter
2, 1 L volumetric. flasks
1, 1 L Erlenmeyer flask
1, 300 mL Erlenmeyer flask
100, 20 mL plastic vials
1-5 mL Oxford pipettor

REAGENTS:

165 µM 4-methyllumbelliferyl-phosphate (mol. wt.= 256.2)
Tris crystals (hydroxymethyl) amino-methane buffer-Base

PROCEDURE:

1. Make 0.1 M Tris buffer: Dissolve 12.114 g Tris in 1 L distilled water. Adjust the pH to 8.5 with HCl (if necessary).

2. Place buffer in a 1 L flask, cover with felt and foil, and autoclave, along with ~200 mL distilled water to adjust the volume after autoclaving.

3. Adjust the pH and volume.

4. Dissolve MUP in 1 L tris buffer (165 µM = .04227 g MUP/L).

5. Pipette ~7.5 mL into 20 mL plastic scintillation vials, cap and freeze immediately. (This is more than will be used: a typical APA run uses 6.3mL of MUP if everything goes smoothly.)

(apacal97.doc)

FLUOROMETER CALIBRATION FOR APA

EQUIPMENT:

Turner 450 Fluorometer fitted with:
1. Quartz-halogen lamp
2. Emission filter: NB460
3. Excitation filter: NB360

12 x 75 mm cuvettes
2, 1-L volumetric flasks
10-100 µL automatic pipettor
100-1000 µL automatic pipettor

REAGENTS:

500 nM 4-methylumbelliferone (MUF)
0.1 M Tris (hydroxymethyl) amino-methane buffer- Base, pH 8.5. (12.114 g/L). (Make 2.5 L)

PROCEDURE:

Calibration of the fluorometer is typically performed at the beginning of the field season, or when a bulb is changed. ****NOTE**The Span knob on the fluorometer must be taped in place. If it is moved, the fluorometer must be recalibrated.**

1. Set up the fluorometer following the procedure in **Alkaline Phosphatase Activity**.

2. Dissolve .0881 g 4-methylumbelliferone (MUF) in 1 L of 0.1 M Tris buffer to make starting stock of 500 µM. MUF is relatively insoluble in water; dissolve first in small amount of methanol then dilute in buffer.

3. Make stock II: Take 1 mL of starting stock and dilute in 1 L of Tris buffer to make 500 nM MUF.

4. Make a triplicate dilution series with stock II:

Add the following amounts to the cuvettes for the 7 triplicate dilutions:

	Tris Buffer	Stock II MUF
1	1975 µL	25 µL
2	1950 µL	50 µL
3	1900 µL	100 µL
4	1850 µL	150 µL
5	1800 µL	200 µL
6	1700 µL	300 µL
7	1600 µL	400 µL

5. Zero the fluorometer using Tris buffer as a blank. Choose a gain and turn the zero knob until the fluorometer reads 000. **You must zero the machine every time you change gains**

6. Mix dilutions by thumping the cuvette near the bottom. Record the fluorescence for each replicate on two appropriate gains (usually 10 and 50).

7. CALCULATIONS:

   a. Calculate MUF concentrations based on the dilutions: (i.e. y' = (25 µL)(500 nM)/(2000 µL) = 6.25 nM)

Dilution	y (µL)	y'=[MUF] (nM)
1	25	6.25
2	50	12.5
3	100	25.0
4	150	37.5
5	200	50.0
6	300	75.0
7	400	100.0

   b. Find the slope (**m**) by dividing the concentration of MUF in each series by the average of the two readings: **m=y'/x**.

   c. Average all slopes (**m**) to get final "door factor".

NOTE:

All glassware should be washed in 2N HCl and rinsed well with distilled water.

Do not contaminate other glassware with MUF; it is highly fluorescent!

(aer97.doc)

AMMONIUM ENHANCEMENT RESPONSE

Note: This procedure last used during the summer of 1995.

EQUIPMENT:

100-1000 µL automatic pipettor
7 300 mL BOD bottles wrapped in foil
6-hole filtration manifold
4.25 cm Whatman GF/F filters
100-1000 µL automatic pipettor (from PPR field box)
7 scintillation vials
Incubator set at lake surface temperature

REAGENTS:

21 µCi ^{14}C-bicarbonate (2.1mL @ 10 µCi/mL)
3 mM NH_4-N stock solution (0.0161g NH_4Cl/100 mL distilled water)
0.01 N HCl
Scintillation fluor (Biosafe)

PROCEDURE:
(Be sure to follow safe radioisotope handling techniques throughout this analysis!)

1. Set up T = 0 filtration tower and pump. Use the first PPR filter tower. Get NH_4-N from refrigerator.

2. Rinse once and fill each BOD bottle with PML (pooled mixed-layer water sample, combined in equal parts from the 3 epilimnetic samples).

3. Transfer ampulated ^{14}C to labeled scintillation vial (under the fume hood).

4. TURN OUT THE LIGHTS. Steps 4 through 11 need to be done in dim light.

5. Remove 0.300 mL water from the seven BOD bottles (three control, three +NH_4, and one T = 0 bottle.) Remove another 0.350 mL from the three +NH_4 bottles. Set T=0 bottle aside.

6. Add 0.350 mL of NH_4-N solution (enrichment = 3.5 µM NH_4-N) to each of the 3 +NH_4 bottles. Change pipette tips.

7. Add 0.300 mL ^{14}C solution (3 µCi) to the three control and 3 +NH_4 bottles, cap and cover with foil. Shake well and place in darkened incubator for 4 hours. This entire procedure should be done as quickly as possible.

8. Add 0.300 mL of the ^{14}C solution to the T=0 bottle, cap and shake the bottle. Filter immediately onto a GF/F filter under the fume hood.

9. Once the sample has filtered completely, rinse the bottle with a squirt of 0.1 N HCl, and filter this rinse. Then rinse the bottle with water and filter this rinse. Rinse tower with 0.1N HCl, and then finally with Milli-Q.

10. Fold filter tightly in quarters, and place in scintillation vial so that scintillation fluor will completely cover it. Dry at 60 - 70 ^{O}C for 24 hours.

11. After the 4 h incubation period, filter contents of the three control and three $+NH_4$ bottles quickly onto GF/F filters. Do this in dim light.(Remember to use vacuum regulator.)

12. Repeat steps 9 and 10.

13. Allow all of the filters to dry overnight. Add 10 mL scintillation fluor to each vial and count in a scintillation counter.

    ** Foil wrapping from bottles, when too scruffy to be reused, should be disposed of in the radioactive solid waste bin.

CLEAN UP:

1. When all samples have been filtered, squirt some acid down the last tower in the line to rinse. When the acid has been pumped out of the line, vent the tower to expel all liquid. Lift towers to drain completely.

2. Rinse BOD bottles and caps three times with hot tap water.

3. Radioactive waste goes into a carboy marked and reserved for radioactive waste.
   NOTE The total radioactivity in each carboy must be known; Record the date when ^{14}C is initially put in the carboy and the date when the final amount of ^{14}C is put in the carboy.

4. Record the amount of radioactivity used in the isotope log book.

CALCULATIONS:

Ratio Calculation: Use the SYSTAT program, CALCAER.CMD.

1. Calculate average cpm's for treatment and control bottles.

2. Subtract cpm of T=0 from the average treatment and control cpms.

3. Divide treatment by control (+NH_4/Control); if value is greater than one, then the phytoplankton are showing nitrogen limitation (increased dark CO_2 uptake after ammonia addition).

Calculation of T-Statistic:

1. Subtract T=0 from all 6 bottles.

2. Find the means (Y_N, Y_C) and the variance (S_N^2, S_C^2), from the 3 +NH_4 and the 3 Control bottles.

3. Calculate the t-statistic (t_S):

$$t_S = \frac{(Y_N - Y_C)}{\frac{\left[(n_N - 1)(S_N^2) + (n_C - 1)(S_C^2)\right]}{(n_N + n_C - 2)} * \frac{(n_N + n_C)}{(n_N)(n_C)}}$$

n_C = # of bottles used for control
n_N = # of bottles used for +NH_4 treatment

4. If $n_N = n_C$, then:

$$t_S = \frac{(Y_N - Y_C)}{\left[\left(\frac{1}{n}\right) * (S_N^2 + S_C^2)\right]^{1/2}}$$

5. d.f. = $(n_N + n_C) - 2$

REFERENCES:

Elser, J.J., M.M. Elser, N.A. MacKay, and S.R. Carpenter. 1988. Zooplankton mediated transitions between N- and P-limited algal growth. Limnol. Oceanogr. 31:1-14.

Vincent, W.F. 1981. Rapid physiological assays for nutrient demand by the plankton. I. Nitrogen. J. Plank. Res. 3:685-697.

Yentsch, C.M., C.S. Yentsch and L.R. Strube. 1977. Variations in ammonium enhancement, an indication of nitrogen deficiency in New England coastal phytoplankton populations. J. Mar. Res. 35:537-555.

(nutenrch97.doc)

NUTRIENT ENRICHMENT EXPERIMENT

(note: This method is currently not used.)

EQUIPMENT:

Water bath and light table on 14:10 h light:dark cycle
18, 300 mL Erlenmeyer flasks (acid washed in 4N HCL and rinsed with distilled water)
100-1000 µL automatic pipettor
125 µm mesh Nitex net

STOCK SOLUTIONS:

0.032 M NH_4 stock solution (1.712 g NH_4Cl/L, distilled H_20)
0.0032 M PO_4 stock solution (0.5574 g K_2HPO_4/L, distilled H_2O)

PROCEDURE:

1. Turn on the water bath (set at current lake epilimnetic temperature) and the light source (set on a 14:10 h timed light:dark cycle).

2. Obtain ~6 L water (1 large and 1 small jug) from epilimnion by pooling the top 3 water depths (100%, 50%, and 25% surface irradiance).

3. Filter ~4 L of the water through a 125 µm Nitex screen to remove zooplankton. Save remaining unfiltered water.

4. Label duplicate flasks for each treatment for each lake: C1,C2,N1,N2,P1,P2,NP1,NP2; rinse once with the filtered lake water, then add 300 mL to each.

5. Add 0.5 mL of the N stock solution to the duplicate N and NP flasks and mix.

6. Change tips, add 0.5 mL of the P stock solution to the P and NP flasks and mix.

7. Mix all flasks well and plug tops with cotton.

8. Place the flasks randomly in the water bath.

9. Take initial chlorophyll samples:

    a. Filter the unfiltered and filtered water from each lake onto GF/F filters

b. record volume (usually 250 mL)

c. freeze

10. Incubate flasks for 4 days, randomizing and mixing the flasks daily.

11. Remove all of the flasks from the water bath.

a. Shake vigorously.

b. Filter desired amount onto GF/F filter.

c. Freeze

d. Record volume filtered for each flask.

12. Measure chlorophyll levels (see **Chlorophyll *a* Analysis**).

REFERENCES:

Bergquist, A.M. 1985. Effects of herbivory on phytoplankton community composition, size structures, and primary production. Dissertation. University of Notre Dame, Notre Dame, IN. U.S.A.

Elser, J.J., Monica M. Elser, Neil A. MacKay and Stephen R. Carpenter. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. Limnol. Oceanogr. 33:1-14.

St. Amand, Ann L., P.A. Soranno, S.R. Carpenter and J.J. Elser. 1989. Algal nutrient deficiency: Growth bioassays versus physiological indicators. Lake and Res. Manage. 5:27-35.

(chlanal97.doc)

CHLOROPHYLL *a* ANALYSIS

EQUIPMENT:

Film canisters
Turner 450 Fluorometer fitted with:
1. Quartz-halogen lamp
2. Emission filter -SC665
3. Excitation filter -NB440

4.25 cm Whatman GF/F filters
12 x 75 mm disposable glass culture cuvettes (**Do not reuse cuvettes!**)
1-5 mL Oxford pipettor
10-100 µL automatic pipettor

NOTE
-**Change filters with fluorometer off!** (Remember that chlorophyll analysis filters are different from APA analysis filters.)
-**Make sure Fluorometer has been calibrated for chlorophyll a** (see **Fluorometer Calibration for Chlorophyll a Analysis**).

REAGENTS:

100% Methanol, spectrophotometric grade
CAUTION - wear gloves whenever you use methanol.
0.1 N HCL

PROCEDURE:

A. **Filtering** (see full Chlorophyll filtering procedure in **Routine Limnological Laboratory Procedure**).

1. For each depth, filter enough water so there is a faint color on the filter. Record volume filtered.

2. Rinse filter tower with distilled water, put filter in film canister, and freeze.

3. For edible chlorophyll, repeat 1 and 2 above, but first filter the sample through 35 µm mesh.

B. **Extraction - DO IN DIM LIGHT and WEAR GLOVES!!**

1. Remove one tray of film canisters from the freezer. Extract chlorophyll by adding 25 mL 100% MeOH to each film canister. (Record extraction volume if different from 25 mL.)

2. Place in refrigerator to extract for exactly 24 hours in the dark.

3 Repeat steps 1 and 2 for all trays that have been in the freezer more than 24 hours.

C. **Fluorometry**

****NOTE**The Span knob on the fluorometer must be taped in place. If it is moved, the fluorometer must be recalibrated.** Calibration of the fluorometer is typically performed at the beginning of the field season, or when a bulb is changed.

1. Insert correct filters in fluorometer while fluorometer is off. (Emission filter -SC665, Excitation filter -NB440), and warm it up for 1 hour .

2. **TURN LIGHTS OUT**. Chlorophylls must be read in low light and samples must be kept cool. Do not remove film canisters from the refrigerator until you are ready to process the samples.

3. Place clean cuvettes into a labeled rack (12 cuvettes per rack). Remove one lake-day of film canisters from the refrigerator.

4. **Shake film canister**, remove the lid, and rinse the pipette tip with 2.5 mL of the sample. Then remove 2.5 mL of sample and place in cuvette.* Repeat for all film canisters.

5. Pipette 2.5 mL of 100% methanol into a cuvette for the blank and use it to zero the fluorometer. Choose a gain and turn the zero knob until the fluorometer reads 000. **You must zero the machine every time you change gains.**

7. Remove the first sample cuvette from the rack, wipe with a Kimwipe, and place in fluorometer. Record the gain and the fluorescence before acidification, $\mathbf{F_b}$. Repeat for all 12 cuvettes in the rack. Readings should be between about 200 and 1000. If not, adjust the gain and re-zero.

8. Acidify each cuvette with 77.3 µL 0.1 N HCl and mix (hold the top of the cuvette securely, then "thump" the bottom several times). Check for condensation on the outside of the cuvettes, and wipe with a Kimwipe if necessary. Wait about 1 min from the acidification of the first cuvette.

9. Record the fluorescence after acidification for all 12 cuvettes. VERY IMPORTANT: **Make sure you read the $\mathbf{F_b}$ and $\mathbf{F_a}$ values for each sample on the same gain**.

10. Remove a new lake-day batch of film canisters from the refrigerator and repeat steps 3-9.

* if particulate matter is present, centrifuge sample for 10 min. and use supernatant.

D. **Clean Up** : **DO THIS UNDER THE HOOD!**

1. Dump methanol solution from cuvettes and film canisters into a metal tray. Place the film canisters and lids in a separate tray. Position them in one layer on the tray with their openings facing up. Leave the trays under the hood overnight to evaporate the methanol.

CALCULATIONS:

Chl *a* (μgL^{-1}) = $(\mathbf{F_b}\text{-}\mathbf{F_a}) * \mathbf{Q}$

Pheoph. (μgL^{-1}) = $((\mathbf{R} * \mathbf{F_a}) - \mathbf{F_b}) * \mathbf{Q}$

Where:

$$\mathbf{Q} = \mathbf{m} * \frac{\mathbf{R}}{\mathbf{(R\text{-}1)}} * \frac{\text{extraction volume}}{\text{filter volume}}$$

m = scale factor (slope)
R = acid ratio
(see **Fluorometer Calibration for Chl. *a*)**

$\mathbf{F_b}$ = fluorescence before acidification

$\mathbf{F_a}$ = fluorescence after acidification

REFERENCES:

Marker, A.F.H., C.A. Crowther, and R.J.M. Gunn. 1980. Methanol and acetone as solvents for estimating chlorophyll *a* and phaeopigments by spectrophotometry. Arch. Hydrobiol. Beih. Ergebn. Limnol 14: 52-69.

Strickland, J.H. and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fish. Res. Brd. Can. Bulletin 167.pp. 201-206.

Holm-Hansen, O. 1978. Chlorophyll *a* determination: improvements in methodology. Oikos 30:438-447.

(chlcal97.doc)

FLUOROMETER CALIBRATION FOR CHLOROPHYLL a ANALYSIS

EQUIPMENT:

Turner 450 Fluorometer fitted with:
1. Quartz-halogen lamp
2. Emission filter -SC665
3. Excitation filter -NB440

12 x 75 mm cuvettes
1, 1 L volumetric flask
1, 1 L Erlenmeyer flask
1, 100 mL volumetric flask
10-100 µL automatic pipettor
100-1000 µL automatic pipettor
Spectrophotometer (665 nm) (Kontron at Trout Lake)

REAGENTS:

1 mg Chlorophyll *a* (from *Anacystis nidulans*)
100% Methanol, spectrophotometric grade
0.1 N HCl

PROCEDURE:

Calibration of the fluorometer is typically performed at the beginning of the field season, or when a bulb is changed. ****NOTE**The Span knob on the fluorometer must be taped in place. If it is moved, the fluorometer must be recalibrated.**

****NOTE** Perform the entire procedure in low light while wearing gloves:**

1. Dissolve 1 mg chlorophyll *a* in 1 L methanol in a 1 L volumetric flask that has been covered with foil to keep out light. Let sit overnight in the refrigerator with a magnetic stirrer.

   NOTE After the chlorophyll has been dissolved overnight, transfer approx. 300 mL of stock from the volumetric flask to a 500 mL jar that has been covered in foil. Make sure that no undissolved chlorophyll is poured into the jar and make Stock I and II from this (see 3.)

2. Set up fluorometer following procedure in **Chlorophyll *a* Analysis**. Let warm up for 30 min.

3. Make stock solutions:

   a. Stock I (1 mg Chl/L): original stock

b. Stock II (5 µg Chl/L): Dilute 0.5 mL of stock I to 100 mL with methanol in a volumetric flask

4. Make dilution series (in triplicate):

Est. Chl (µg/L)	Actual Chl (µg/L)	Methanol (mL)	Stock I (mL)	Stock II (mL)
1.0		2.000	-	0.500
5.0		0	-	2.500
10.0		2.475	0.025	-
20.0		2.450	0.050	-
50.0		2.375	0.125	-
100.0		2.250	0.250	-
200.0		2.000	0.500	-
500.0		1.250	1.250	-

5. AT TROUT LAKE: Find concentration of chlorophyll in the stock solution. Measure absorbance of the stock I solution at 665 nm on the spectrophotometer (warm up spec for 20 min.). Calculate actual chlorophyll concentration of stock I from the specific absorption coefficient of chlorophyll in methanol (use 75 liter/g/cm for absolute methanol (Riemann, 1980)). Divide the absorbance by 75 to get g Chl./L.

6. Divide the actual conc. of stock I (from Step 5.) by the estimated Chlorophyll conc. of stock I (i.e. 1 mg Chl/L) to calculate the conversion factor (percentage of estimated Chl. conc. of stock I). Multiply the conversion factor by the **Estimated Chlorophyll** concentrations in the dilution series (see table in Step 4.) to calculate the **Actual Chlorophyll** concentrations. Enter these concentrations in the table in Step 4.

7. Zero the fluorometer using 2.5 mL methanol as the blank. Choose a gain and turn the zero knob until the fluorometer reads 000. **You must zero the machine every time you change gains.**

8. Record fluorescence units before acidification ($\mathbf{F_b}$) and after acidification ($\mathbf{F_a}$), with 0.1 N HCl, for each dilution series, following the procedure in **Chlorophyll *a* Analysis**. Measure $\mathbf{F_b}$ and $\mathbf{F_a}$ on as many gains as possible and reasonable for each series.

9. Obtain the acid ratio (**R**) by calculating $\mathbf{F_b/F_a}$ for each recorded value on each gain. If noticeable differences are noted between gains, then calculate mean ratios for each gain. Otherwise, use the mean for all gains for the final calculations.

10. Find **x** for each reading on each scale (where **$x=F_b-F_a$**) and average the triplicates. Calculate the slope **m** (**m=y/x**), where **y=Actual Chl** in µg/L in the table in Step 4.

11. Calculate **Q** factors for each scale at a variety of commonly used filter volumes (use extraction volume of 25 mL for all) and put in table form, or on a spreadsheet:

$$\mathbf{Q = m * \frac{R}{(R-1)} * \frac{extraction\ volume}{filter\ volume}}$$

where:

m = slope (mean value from each scale)
R = ratio (either grand mean or mean for each scale)

REFERENCES:

Riemann, B. 1980. A note on the use of MeOH as an extraction solvent for Chlorophyll *a* determination. Arch. Hydrobiol. Beih. 14: 70-78.

(C14ppr97.doc)

^{14}C-PRIMARY PRODUCTIVITY

EQUIPMENT:

Field:

PPR float with line and clips for hanging bottles at selected depths
PPR field boxes containing:
- 18, 60 mL reagent bottles w/ glass stoppers, + extra bottles and tops.
- 2, 100-1000 µL automatic pipettor and several tips
- lab gloves and plastic apron
- plastic bag for used gloves and pipette tips
- extra clips for hanging bottles
- water pump

Lab:

6-tower vacuum filter apparatus
4.7 cm Whatman GF/F filters
Scintillation vials with labeled caps (label includes lake, day, "D" or "L" for dark or light bottle, and depth, respectively. (e.g.: "L145 L1"; "W224 D6")

REAGENTS:

10 µCi ^{14}C-bicarbonate (2 µCi mL^{-1})
Dichlorophenol-dimethyl urea (DCMU) saturated solution
0.1 N HCL
Scintillation fluor (Biosafe)

PROCEDURE:
(Observe safe radioisotope handling techniques at all times during this analysis!)

A. In lab (morning, before going to the field)

1. Transfer ^{14}C from ampule to labeled scintillation vial with a disposable pipettor (under the fume hood). Put scintillation vial in the field box.

2. Replenish supply of lab gloves, pipette tips, and DCMU in the field box.

3. Remember to include the PPR float and the field boxes (check contents with list) in the items loaded into the field vehicle(s).

B. In field

1. Rinse and fill 3, 60 mL BOD bottles with water from each depth, corresponding to 100, 50, 25, 10, 5 and 1% of surface irradiance. Avoid getting air bubbles in bottles. TRY TO KEEP BOTTLES IN THE DARK AS MUCH AS POSSIBLE.

2. Pipette 250 µL of water from each bottle (using the "^{14}C pipettor").

3. Pipette an additional 500 µL of water from the 6 "dark" bottles using the "DCMU pipettor" (the "dark" bottles are used as a t = 0 control).

4. Using the "DCMU pipettor," add 500 µL DCMU to the "dark" bottles to kill the phytoplankton. (always done before ^{14}C addition.) **It is important not to contaminate "light" bottles with DCMU!** Dark bottles are labeled, and are used only as dark bottles.

5. Using the "^{14}C pipettor," Pipette 250 µL of ^{14}C into each of the 18 bottles, starting with the "dark" bottles to ensure there is enough isotope for controls.

Summary of subtractions and additions:

	light bottles	**dark bottles**
remove	250 µL	750 µL (250 + 500)
add DCMU	-	500 µL
add ^{14}C	250 µL	250 µL

6. Replace stoppers and invert bottles 2 or 3 times to mix. Ensure that stoppers are well-seated, so they don't come out. It often helps to twist the stopper as you push it into the bottle.

7. Suspend bottles at appropriate depths for incubation. **Record incubation start time**.

8. AFTER 6 HRS: Remove bottles from water and place in carrying case until ready to filter (filtering should be done promptly after removal of bottles from water). Record incubation finish time. (Incubations usually go from ~9:30am-3:30pm)

C. In lab (afternoon)

1. Have ready

   a. Flask used only for collecting ^{14}C waste

b. Filter towers equipped with 4.7 cm GF/F filters. Separate towers should be used for light and dark (DCMU) bottles.

c. Scintillation vials, with caps labeled for all samples.

d. A full squirt bottle of 0.1 N HCl and a full squirt bottle of Milli-Q

TURN OFF THE LIGHTS - THE REST OF THE PROCEDURE SHOULD BE DONE IN DIM LIGHT!

2. Prepare 3 totals:

a. Add 10 mL scintillation solution (Biosafe) and 100 µL 1 N NaOH to 6 vials (label on cap should include lake, day, "TOT" ,and depth id).

b. Remove 250 µL from one of the light bottles from each depth and add it to the proper vial. These vials are for calculating the total amount of ^{14}C added to the bottles.

c. Tightly cap the total vials and put aside for later analysis with the scintillation counter

3. Samples (process samples in the designated ^{14}C fume hood):

a. Empty the entire BOD bottle into the appropriate "light" or "dark" filter tower. Record volume if entire bottle is not filtered.

b. Once the sample has filtered completely, rinse the bottle with a squirt of 0.1 N HCl, and filter this rinse. Then rinse the bottle with water and filter this rinse. Rinse tower with 0.1N HCl, and then finally with Milli-Q.

c. Remove filter by folding it in quarters and place it at the bottom of the appropriate scintillation vial. Filter should be compact enough in the bottom of the vial to be completely covered by the scintillation fluor (which fills half of the vial).

d. Dry at 60-70°C for 24 hours.

e. After drying filters, add 10 mL liquid scintillation solution to vials and count in scintillation counter (see Scintillation Counting Procedure).

D. Clean up:

1. When all samples have been filtered, squirt some acid down the last tower in the line to rinse. When the acid has been pumped out of the line, vent the tower to expel all liquid. Lift towers to drain completely.

2. Rinse BOD bottles and caps three times with hot tap water.

3. Radioactive waste goes into a carboy marked and reserved for radioactive waste. **NOTE** The total radioactivity in each carboy must be known; Record the date when ^{14}C is initially put in the carboy and the date when the final amount of ^{14}C is put in the carboy.

4. Empty the remaining amount of ^{14}C from the scintillation vial taken into the field into the radioactive waste carboy. Discard the vial in the dry radioactive waste bag.

5. Record the amount of radioactivity used in the isotope log book.

CALCULATIONS:

Use the SYSTAT command file CALCPPR.CMD to calculate primary productivity according to the following equation:

$$\text{mg C}*\text{m}^{-3}*\text{h}^{-1} = \frac{(\text{CPMs} - \text{CPMb}) * (\text{Vinc}/\text{Vfil}) * (\text{A}) * (1.05)}{(\text{DPMt}) * (\text{Eff}) * (\text{T})}$$

where:

CPMs = counts per minute for sample
CPMb = counts per minute for DCMU control
Vine = volume (mL) incubated
Veil = volume (mL) filtered
A = total C in sample (in mg C/m^3), calculated from sample alkalinity
1.05 = isotope discrimination factor
DPMt = disintegrations per minute of total amount of ^{14}C added to each bottle
Eff = efficiency of scintillation fluor calculated from internal standards for each sample
T = length of incubation (h)

2. Use the method in Appendix III, along with measurements of solar radiation* and light extinction from the weekly light profiles, to calculate daily production of the photic zone and the mixed layer (see Carpenter et al., 1986).

* see **Pyranograph Method**

REFERENCES:

Carpenter, S.R., M.M Elser and J.J. Elser. 1986. Chlorophyll production, degradation, and sedimentation: Implications for paleolimnology. Limnol. Oceanogr. 31:112-124.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167:267-279.

Legendre, L., S. Demers, C.M. Yentsch, and C.S. Yentsch. 1983. The ^{14}C method: Patterns of dark CO_2 fixation and DCMU correction to replace the dark bottle. Limnol. Oceanogr. 28: 996-1003.

(C14amp97.doc)

AMPULATING ^{14}C

EQUIPMENT:

1 L volumetric flask
100 mL volumetric flask
5 mL and 20 mL glass ampules
1-5 mL Oxford pipettor
100-1000 µL Eppendorf pipettor
pH meter
torch or gas flame for sealing ampules
forceps
protective wear for radioactive materials

REAGENTS:

2 mCi ^{14}C-bicarbonate (these instructions use 1 mL @ 2 mCi/mL)*
Na_2CO_3
10 N NaOH

PROCEDURE:

1. Prepare the buffer: dissolve 0.15 g of Na_2CO_3 in one liter of Milli-Q. Titrate to pH 10.5 with 10N NaOH.

2. Perform necessary dilutions to desired working activity: The ^{14}C provided by the manufacturer comes in a sealed ampule. Break open the ampule (carefully!). This 14C-bicarbonate solution in the ampule is designated Stock A. Stock A must be diluted to make Stock B, which is used in AER (Ammonium Enhancement Response) studies. Stock B is diluted to make Stock C and used for PPR (primary productivity) studies.
   a. In these instructions, only 0.5 mL of the 1 mL original 14C-bicarbonate is used. The remainder must be ampulated and refrigerated.

To Make	Use	Volume Stock (mL)	Volume Buffer (mL)	Final Volume Stock (mL)	[Final Stock] (µCi/mL)	Total Activity Stock (µCi)
B	A (2000 µCi/mL)	0.5	99.5	100	10	1000
C	B (10 µCi/mL)	50	200	250	2	500

3. Ampulating: The AER and PPR procedures use different concentrations of ^{14}C-bicarbonate solutions. The following guide includes the number of ampules needed for the summer for:

   a. AER: The number of ampules specified in the table below is enough for 5 sampling days (one sampling day every 4 weeks through the summer) for 4 lakes.

   b. PPR: East Long, West Long, Paul, and Peter Lakes: 11 mL per sampling day per lake (0.5 mL per sample, triplicate samples per depth, 6 depths per lake plus some extra). The number of ampules specified in the table below is enough for 5 sampling days (one sampling day every 4 weeks through the summer) for 3 lakes.

   c. Ampulate remaining ^{14}C- and refrigerate ampules.

   d. These stock volumes and numbers of ampules must be recalculated each year depending on the frequencies with which AER's and PPR's will be done over the summer .

Procedure	Use Stock	Volume/ ampule (mL)	# Ampules	Ampule Size (mL)	Total Volume Used (mL)	Total Activity (µCi)	Activity per Ampule (µCi)
AER	B (10 µCi/mL)	2.5	20	5	50	500	25
PPR							
Paul East Long West Long	C (2 µCi/mL)	11	15	20	165	330	22
Peter	C (2 µCi/mL)	14	5	20	70	140	28
Extra C	C (2 µCi/mL)	15	1	20	15	30	30
						1000	

REFERENCES:

Vollenweider, R.A. 1969. A Manual on Methods for Measuring Primary Production in Aquatic Environments. IBP Handbook No. 12. Davis, Philadelphia. 213 pp.

Strickland, J.D. and T.R. Parsons. 1968. A practical handbook of seawater anal. Bull. Fish. Res. Bd. Can.167. pp.201-206.

(pyro97.doc)

PYRANOGRAPH

EQUIPMENT:

RECORDING:

Weathertronics mechanical pyranograph
176 - hour (7 - day) pyranograph chart paper
Driorite desiccant

CUTTING UP:

Pen knife
Mettler balance (0.0001 g precision)

PROCEDURE:

A. Recording:

1. Change paper and desiccant every 6 days. Be sure to label each chart with beginning and end dates.

2. Check to make sure the pyranograph is level, using the bulls-eye level on the machine.

B. Cutting Up: The general idea is to find the area under the chart, which is a measurement of the surface irradiance hitting the pyranograph, in einsteins/m^2.

1. Photocopy each Pyranograph chart, and archive the originals.

2. Cut a known area (e.g. 10 cm^2) of each photocopied page to be massed as a reference.

3. Cut out the photocopied charts, following the pyro pen line as closely as you are able (or have patience for.) (Be sure to label each day!)

4. For PPR days, mark start and end times of the PPR experiment on the chart.

5. Mass the reference squares on the Mettler balance. Calculate the conversion factor by dividing this mass by the total number of squares massed (chart mass in grams/chart area in cm^2)

6. Mass each day's chart, and record the mass in g.

7. In addition, for PPR days cut the chart area representing the experiment time. Mass and record this area separately.

8. Calculate surface irradiance:

surface irradiance(einsteins/m^2/day) = mass of curve(g) ***** (13.892 / **b**)

where:

b = conversion factor (chart mass(g)/chart area(cm^2), see step 5)

9. Record data in DAYSOL9?.XLS (daily data) and in INLITE9?.XLS (PPR data)

(phyto97.doc)

MOUNTING OF PERMANENT PHYTOPLANKTON SLIDES USING HPMA

EQUIPMENT:

Bunsen burner
Beaker tongs
Ice bath
Pyrex beakers (150 mL)
2 Dropper bottles (amber, 30 mL)
Mixed ester cellulose membrane filters (.45 :m, 25mm, plain white)
Glass slides (frosted 1 end, 3" x 1", a mm thick)
Nalgene polypaper pressure-sensitive labels (7/8" x 7/8")
Glass cover slips (25 mm x 25mm)
Full view series support/drying racks (102 pin)
Graduated cylinder (10-50 mL)
Dumont forceps
Glass or Polysulfone filtration towers
Rubber stoppers (#8, 1-hole, 1 for each tower)
Filtration manifold
Vacuum pump (plus appropriate plumbing, app. **50 PSI**)
Drying oven (60 ^{o}C, **not** forced air)
Fume hood

REAGENTS:

HPMA (2-hydroxypropyl methacrylate)
Catalyst (azo-bis-iso-butyronitrile)
Iodine
Glutaraldehyde (25%, general grade)
Distilled water

CAUTION: Store HPMA and catalyst in refrigerator.
Keep Glutaraldehyde under hood

METHOD:

A. SAMPLE:

1. Add approximately 2-3 mL of glutaraldehyde to the pooled mix layer water sample and refrigerate. Keep dark.

2. Remove sample from refrigerator and let warm to room temperature before mounting.

B. RESIN:

1. Prepare ice bath in plastic tub.

2. Measure 25 mL of HPMA and 0.025 g of catalyst into 150 mL beaker

   Deal with HPMA under hood and use gloves for both HPMA and catalyst.

3. Under hood, light Bunsen burner and set to high flame.

Heat HPMA (with catalyst added) until you see density currents starting to form. Cool mixture by swirling in ice bath, and return to flame. **DO NOT LET MIXTURE BOIL!!!!** Keep heating and cooling, alternately, until the thickness of Karo syrup. Make sure the mixture is cool when it reaches this point or it will polymerize further. Transfer to a clean, glass jar for storage until use. The entire procedure takes 1/2 - 1 hour, depending on how brave you are. **CAUTION: THIS REACTION IS EXOTHERMIC ONCE IT REACHES A CERTAIN TEMPERATURE AND WILL TAKE PLACE ALMOST EXPLOSIVELY IF YOU LET IT GET TOO HOT. THE FUMES ARE TOXIC. KEEP WATER OUT OF THE PRE-POLYMER.**

NOTE: Wash beakers in ethanol by letting them soak for 24-48 h twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin.

4. Fill 2 amber dropper bottles with resin. Add crystalline iodine to one of the bottles until the resin is nearly opaque. The iodine-resin will be slightly thicker than normal resin. (Resin is light sensitive -- be sure to cover the extra resin with foil.)

C. SLIDES: Make 3 slides for each sample and shake sample well.

1. Put membrane filters onto filtration bases and wet with distilled water. Drain excess water through filter. If filter has any opaque areas (very white when wet), replace with another filter.

2. Assemble towers.

3. Measure out 1-20 mL of sample into graduate cylinder. Choose sample volume so that each field at 200X contains approximately 20-30 cells.

4. Add sample to the tower and open valve. Filter sample until water just clears the filter surface. Close valve and remove filtration tower just **after** the water disappears form the inner edge of the tower.

5. Place filter, FACE down, on a cover slip. Be careful to avoid bubbles under the filter.

6. One or two slides:

   a. 2 slides: Add 1 - 2 drops of clear resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.

   b. 1 slide: Add 1 - 2 drops of iodine-resin to the back of the filter, and rotate the cover slip until the resin covers the back of the filter.

7. Place cover slips on the drying rack and place in drying oven for 12 - 24 hours.

8. Remove cover slips from oven. Add 1 - 3 drops of resin to the filter side of the cover slip and attach to a labeled slide. Add as little resin as possible to cover the filter surface! The less resin, the faster it will polymerize and the better the prep.

9. Put slides in the oven and let polymerize for approximately 24 hours. If the resin is not completely polymerized, replace and heat for as long as 2 - 3 more days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate! AND BELIEVE ME IT'S A MESS!

D. COUNTING PROCEDURES: (1988-)

1. Count a minimum of 15 fields at 200X, and a minimum of 300 cells total.

2. On 400X, count a minimum of 10 fields and 100 cells of miscellaneous microflagellates and small species (<7 microns GALD).

3. Measure as many cells as counted, or 20-30 individuals and/or colonies.

4. Calculations: Use ALGAE88G.BAS (see program disk) to calculate biovolumes, volumes and concentrations.

E. COUNTING PROCEDURES: (1984-1987)

1. Samples preserved in Lugol's solution and settled in towers for 3 days.

2. Samples then settled (–1 h) and counted in Sedgewick Rafter cells.

3. Counted at 200X only: 3 short passes, or a minimum of 10 fields.

(zoop97.doc)

ZOOPLANKTON SAMPLING AND COUNTING PROCEDURE

A. SAMPLE COLLECTION:

Zooplankton are sampled weekly in each lake using a pooled duplicate haul for a preserved count, and a single haul for a live count. The same zooplankton net should be used throughout the field season. It is important to raise the net at a consistently steady rate (3 seconds per meter) when collecting each sample.

1. Take 2 vertical tows (depths shown below) with an 80 µm mesh Nitex net and pool them into an empty 4 oz. jar. Add an amount of Lugols which turns the sample a brandy color (approx. 3 mL). Make sure the cap is on tight and store the sample in the dark. Periodically check the samples for lightening of color, in which case more Lugols should be added. See the following Lugols recipe.

Tow depths:

LAKE	DEPTH
Paul	8m
Peter	12m
East Long	12m
West Long	12m
Central Long	4m
Tuesday	12m

2. Take 1 additional vertical tow (live count) from the same depth, and place in an empty 4 oz. jar. At the lab, pour the entire sample into a counting tray and count the number of *Conochilus* colonies.

NOTE Measure *Conochilus* colony size (20 - 30 colonies), and count the number of individuals per colony in each lake as soon as they appear in the lake (randomly select 3-5 colonies and count the individuals from each). Do this monthly thereafter in each lake, if and when *Conochilus* are present.

B. COUNTING PROCEDURE:

1. Wash the sample, using an 80 µm Nitex screen and tap water, into a counting dish with 1.3 cm x 1.3 cm grids (wear gloves). Normally, the sample should be split to reduce the zooplankton concentration (using a Folsom plankton splitter). A good rule of thumb is to split the sample until there are in the range of 200 - 500 total zooplankton per grid square. Be sure to note on the counting form the number of times the sample was split.

2. Using a binocular dissecting scope, count and identify all zooplankton in randomly chosen grids within the center 16 grids, on 25X (excluding the larger zooplankton: *Daphnia*, *Holopedium*, *Mesocyclops,* and *Epischura*, see step 4.). See the separate Taxonomic Key of Zooplankton in the Cascade Lakes for genus/species identification.

3. Continue counting grid squares until the standard error of the total number of animals per square is less than 10% of the mean (standard error = standard deviation/$\sqrt{n}$, where *n* = number of grid squares counted). Count a minimum of 3 grid squares. **NOTE** For the smaller zooplankton that are less abundant, it may be better (more accurate and not too time consuming) to count the entire tray, or 1/2 of the tray, rather than individual grid-squares.

4. Count all the larger zooplankton, *Daphnia, Holopedium, Mesocyclops, and Epischura,* in the entire tray, on 12X.

5. Measure the lengths of the copepods and cladocerans (except *Daphnia* (see Step 6.)) on a known magnification until the standard error is less than 10% of the mean for each taxa. The only rotifer which should be measured is *Asplanchna*. Measure a minimum of 8 lengths per taxa.

6. Randomly choose ~50 daphnids. Measure lengths, and identify to species to determine the proportion of each species present.

7. After completing the count, return the sample to its jar and prepare the sample for long term storage by adding an equal amount of formalin as sample. See the following formalin recipe.

8. Calculate the total number of zooplankton per 2 net hauls (Tot/2):

   Tot/2 = (total # of animals in entire tray) * 2 **d** {for larger zoop.}

   or, for all other zoop.:

   Tot/2 = (ave. # of animals per grid square) * 39.6 squares/tray * 2 **d**

   d = number of times sample was split using Folsom splitter

9. Data from the counting sheet is entered into lake specific Excel templates ZOOP?9?.XLS (e.g. ZOOPL9?.XLS is the template for Paul Lake). Note that the net efficiencies should be calculated and entered before the count data (see **Calculation of Zooplankton Net Efficiency**).

C. PRESERVATIVE RECIPES:

1. Lugols:

5 g Iodine
10 g Potasium iodide
10 mL Glacial acetic acid
100 mL distilled water

2. Formalin: (store in refrigerator)

To make 3 L of 8% sugared buffered formalin solution:

650 mL of 37% formaldehyde
2350 mL of distilled water
500 g sugar
6 g Borax

3. Hoyers: (mounting medium for slides)

30 g Gum arabic
100 g distilled water
200 g chloral hydrate
20 g glycerine

Can make partial recipe. Use heat to dissolve ingredients in distilled water.

REFERENCE

Downing, John A. and F.H. Rigler. 1984. A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters. Blackwell Scientific Publications. Oxford, England.

CALCULATION OF ZOOPLANKTON NET EFFICIENCY

The efficiencies of the zooplankton net (the same net should be used throughout the field season) should be determined every year, for each lake, and for each of the functional groups listed in Appendix I, EFFIC9?.XLS. **The efficiencies are then averaged with past years efficiencies for use in biomass calculations per lake.**

A. Take 2 vertical hauls at the weekly zooplankton tow depth, and pool and preserve them (see **Zooplankton Sampling and Counting Procedure**).

B. Take samples from the surface to the tow depth, at evenly spaced intervals (usually every 1 m), using the 12 L Schindler-Patalas trap, and pool them into a 500 mL jar (rinse well between samples using tap water). Begin the sampling at the surface and work down, making sure the trap goes straight down as it is lowered (do not try on windy days since it is difficult to get the trap lowered to the desired depth without it closing first).

C. Count the pooled vertical hauls and the pooled Schindler samples using the method for the weekly zooplankton samples (see **Zooplankton Sampling and Counting Procedure**). It is not necessary to take length measurements for either sample. **Note that when counting, the zooplankton can be grouped according to the list in Appendix I, EFFIC9?.XLS.**

CALCULATIONS:

a. **Apparent density** (vertical hauls) - Divide the number of animals found in **1** haul by the volume of water swept by the net:

App Den = # animals/VolH
VolH (m^3) = (net radius)^2 * haul depth * π
net radius = 0.15m

b. **Actual density** (Schindler Trap) - Divide the number of animals found in the pooled Schindler samples by the volume of water sampled:

Act Den = # animals/VolS
VolS (m^3) = 12L * # samples taken * $1m^3/1000L$

c. **Net Efficiency** = App Den/Act Den

D. Enter the efficiencies into EFFIC9?.XLS The efficiencies used in all zooplankton calculations are the average efficiencies of all years represented in EFFIC9?.XLS. Enter these averages into the lake specific templates ZOOP?9?.XLS (e.g. ZOOPL9?.XLS is the template for Paul Lake).

(daphb&d.doc)

CALCULATION OF DAPHNID BIRTH, DEATH AND GROWTH RATES

From 1988-1990, we sampled intensively to obtain accurate estimates of birth, death and growth rates of daphnids in Paul, Peter and Tuesday (1988 only) Lakes. We took weekly samples in each lake, consisting of duplicate hauls from 3 different stations in each lake from the depth the routine zooplankton hauls were taken.

SAMPLING PROCEDURE:

1. The three stations in each lake were chosen across ~15 m over the deepest portion of the lakes on the transect lines.

2. The duplicate hauls at each station were pooled into an empty jar and then washed, with filtered lake water through an 80 µm mesh net into a jar of cold 4% formaldehyde solution (see **Zooplankton Sampling and Counting Procedure**). The jars were stored in a small cooler with ice, brought back to the lab and stored in the refrigerator.

3. The samples were processed the same day they were taken. We found that the sooner the samples were processed, the fewer daphnids exploded.

COUNTING PROCEDURE:

1. The whole sample was concentrated into the counting dish. If a large amount of Daphnia were found in the sample (>~500), the sample was split with a Folsom plankton splitter.

2. The total number of Daphnia in the dish was obtained by scanning and counting the whole sample on 12X.

3. Starting at the top left hand corner, the dish was moved in a set pattern and every animal coming into view was measured and its eggs or neonates were counted. If the animal was exploded, it was recorded as such, and the length measurement was kept, but the animal was <u>not</u> used for any other calculation.

4. This continued until a minimum of 25 animals with eggs was found, or a maximum of about 50 animals was measured per replicate.

CALCULATIONS:

For the following calculations, values for MBS,RR, and E_a were averaged from the 3 replicates taken for each lake.

1. Mean brood size: MBS

   MBS = Average brood size of females containing 1 or more eggs

2. Reproductive ratio: RR

$$RR = \frac{\text{Total number of females with a brood}}{\text{Total number of females of reproductive size}}$$

3. Egg ratio: E_a

$$E_a = MBS * RR$$

4. Egg development time: D (Lei and Armitage, 1980)

$$\ln D = \ln a + T(\ln b) + T^2 \ln c$$

We use the values Lei and Armitage found for field data, using *D. ambigua* within the temperature range of 10-30°C:

$$\ln D = 3.4898 - .1796T + .0024T^2$$

For migrating *Daphnia* in stratified lakes such as ours, we estimate that the adults spend 17 h at 6°C in the deeper waters, and 7 h in the epilimnion at ambient lake temperatures (pers. obs., M.L. Dini).

5. Instantaneous birth rate: b (DeMott, 1983)

$$b = \frac{\ln(E_a + 1)}{D}$$

For migrating Daphnia in stratified lakes: which spend 71% (17 h) of each day in the hypolimnion and 29% (7 h) in the epilimnion:

$$b = \frac{.71}{D_6}(\ln(E_a + 1)) + \frac{.29}{D_S}(\ln(E_a + 1))$$

D_6 = Development time at 6 °C

D_S = Development time at epilimnetic temperature

6. Population growth rate: r , over time interval 0 to t

$$r = \frac{\ln N_t - \ln N_0}{t}$$

7. Death rate: d

$$d = b - r$$

REFERENCES:

DeMott, W.R. 1983. Seasonal succession in a natural *Daphnia* assemblage. Ecol. Monogr. 63:1949-1966.

Hoenicke, R. and C.R. Goldman. 1987. Resource dynamics and seasonal changes in competitive interactions among three cladoceran species. J. Plank. Rsch. 9:397-417.

Lei, Chi-Hsiang and K.B. Armitage. 1980. Growth, development and body size of field and laboratory populations of *Daphnia ambigua*. Oikos 35:31-48.

Vanni, Michael J. 1986. Competition in zooplankton communities: Suppression of small species by *Daphnia pulex*. Limnol. Oceanogr. 31:1039-1056.

(dielsamp.doc)

DIEL SAMPLING PROCEDURE(a short course)*

EQUIPMENT:

144, 125 mL sample jars.
Labels include LAKE DAY TIME DEPTH REP (e.g. "W122 MID 12m A;"
Choose a different color label tape for each lake, to make bottle-sorting easier
2 zooplankton sampling cups with 80µm mesh
1, 12 L Schindler-Patalas clear Plexiglas zooplankton trap
Appropriately-marked 1/4 inch braided nylon ropes, tied securely to the Schindler Trap hook.
Flashlight (for night sampling)
Squirt bottle

REAGENTS: Lots and lots of 8% formalin solution (~10 L per sample date)

PROCEDURE:

1. Sample times should be 12 hours apart: noon and then midnight or midnight and then noon, whichever is easier.

2. Check knot on the Schindler Trap attachment hardware (usually an O-clamp). If the rope comes undone in the water, the horrendously expensive Schindler Trap will become one with the profundal benthos for all eternity.

3. Sample from the surface down. Sample depths (and therefore the "appropriate markings" for the ropes referred to earlier) are:

East Long, West Long, and Peter Lakes	Paul Lake
0 m	0 m
1 m	1 m
3 m	3 m
6 m	5 m
9 m	7 m
12 m	8 m

4. Pool two Schindler hauls from each depth into the sample jar for that depth, making sure the jar is less than half full with sample so that volume can be doubled with 8% formalin preservative.

5. Samples are taken from three sites (approximately 5 m apart) over the deep hole of each lake.

6. Back at the lab, double the sample volume in each and every jar with cold 8% formalin solution to preserve the zooplankton therein.

7. Clean the Schindler trap sock and zooplankton cups with a strong stream of water and a gentle rubbing with the fingers.

CARE AND FEEDING OF YOUR 12-L SCHINDLER TRAP

1. The threads of the O-clamp (to which the rope is securely tied) should occasionally be treated with WD40, especially at the end of the season.

2. If the seams of the trap develop leaks, these are best caulked with epoxy or silicone.

* Consult previous editions of this manual for a long but entertaining version of this methodology.

DIEL SAMPLE ANALYSIS

1. If chaoborids are present, they are usually located at the surfaces of preserved samples. All larvae (all instars too) should be counted by eye and then poured off, leaving the crustacean zooplankton at the bottom of the jar.

2. Preserved zooplankton samples are counted on a binocular dissecting scope at 25X, in a square-gridded petri dish.

3. Count juvenile *Daphnia pulex*, adult *D.pulex*, juvenile *D.rosea*, adult *D.rosea*, *Bosmina*, and copepods. *Daphnia pulex* with a body length less than 1.4 mm can be considered juveniles; *D. rosea* smaller than 1.1 mm should be considered juveniles. If one doesn't care to distinguish these species, the lower of these two values should serve as the juvenile-adult cutoff.

4. Count 1/4 grid at a time, carefully moving the dish back and forth under the scope so as to prevent zoops moving from one square into another.

5. Dense samples (> approx. 80 animals) are subsampled with the Folsom plankton splitter prior to counting.

6. Raw data are entered into a final data file with the following format: Lake$ day time$ aDpulA aDpulB aDpulC jDpulA jDpulB jDpulC aDrosA aDrosB aDrosC jDrosA jDrosB jDrosC BosA BosB BosC Copavg

where: time$ = "D" or "N" for "day" or "night," aDpul? = adult *D.pulex* for tow A,B,and C; jDpul? = juvenile *D.pulex* for tows A,B, and C; Bos? = *Bosmina* spp for tows A,B, and C; and Copavg = the average number of copepods in tows A,B, and C.

(chao97.doc)

CHAOBORUS **SAMPLING AND COUNTING PROCEDURE**

SAMPLE COLLECTION:

1. Sample all lakes biweekly during the summer (using the same net throughout if possible): take 3 vertical tows from 12 m from each lake (8m in Paul Lake) with a 147 µm mesh Nitex net after dusk, and place each tow into a separate jar.

2. Add Lugol's solution to sample immediately after returning to the lab. The sample should be the color of brandy.

COUNTING PROCEDURE:

1. For each of three samples from each lake:

   a. Count total number of *Chaoborus* in sample.

   b. Randomly choose ~35 animals and identify to species (see following figure) (Roth, 1967). Measure the head capsule length to determine the instar (von Ende, 1982), as well as the body width at the 3rd segment and the body length .

   2. Estimate the individual mass from the length-weight regression determined for the *Chaoborus* found in our lakes:

      log(biom) = 1.189 log(vol) - 8.644

   where:

      $vol = \pi * (W/2)^2 * L$

   W = width
   L = length

NOTE:

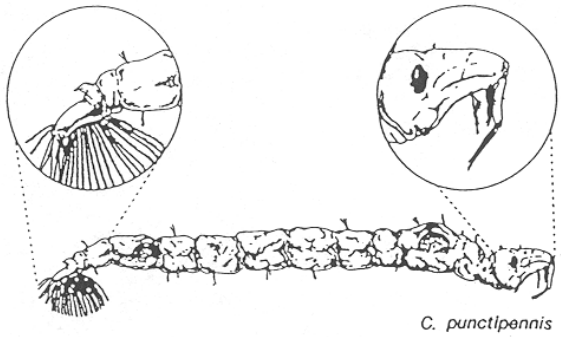
1. 1st instars are small and can be overlooked easily. Care should be taken to include them adequately in the counts and length measurements (the width remains the same for 1st instars so it is only measured in the initial samples). Also, we have found it impossible to identify 1st instars to species.

2. Pupae are excluded from the length, density, and biomass estimates.

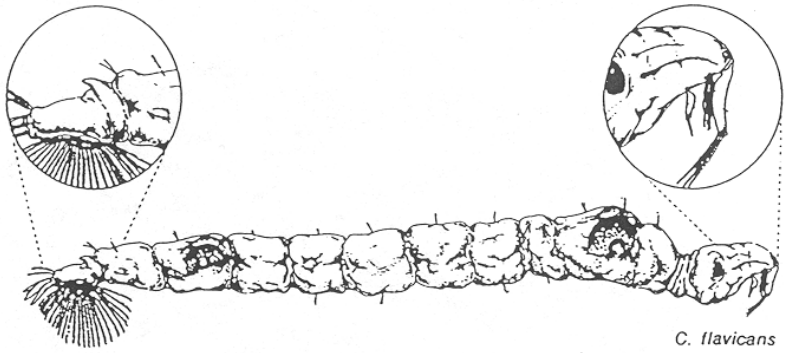
   4. The net efficiency for *Chaoborus* was estimated in 1989 to be .575 for a 3 m tow, and was assumed to be relatively similar for all years (1985-1989), (see Appendix II). In 1993 we switched to 12 m tows (8m in Paul). Efficiencies will be calculated in 1994.

Chaoborus spp. Larvae *

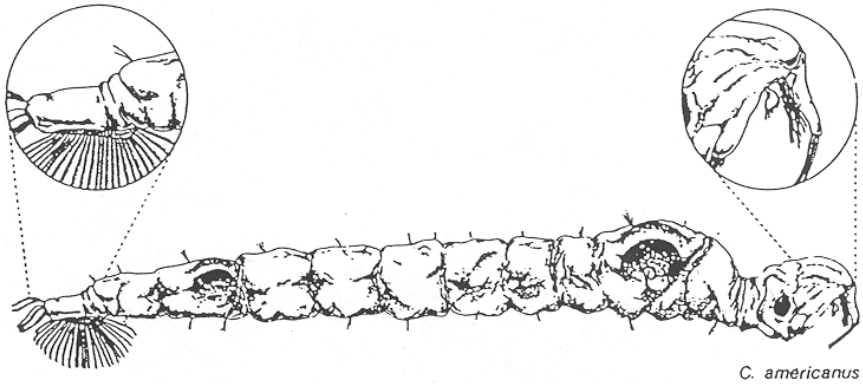
1mm Insets 1000x



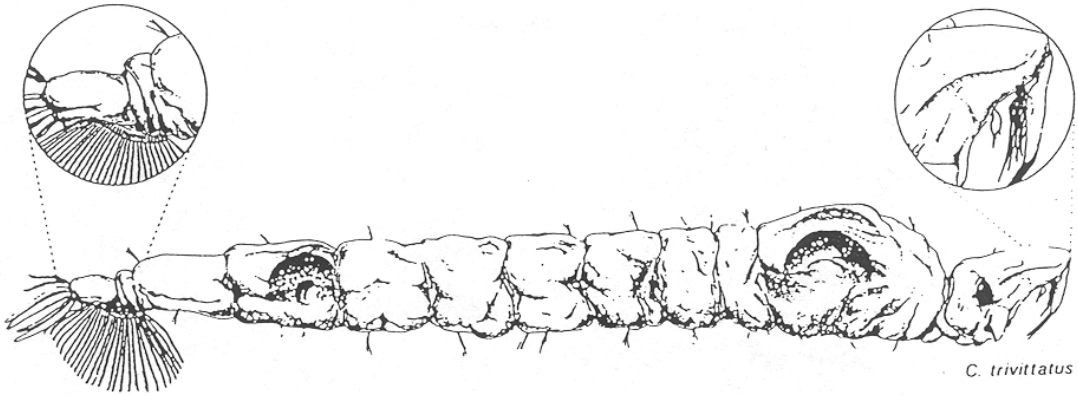
C. punctipennis



C. flavicans



C. americanus



C. trivittatus

* Courtesy of Carl von Ende

CALCULATING A LENGTH-WEIGHT REGRESSION FOR ESTIMATING *CHAOBORUS* BIOMASS

In the summer of 1988, we identified, measured, and weighed *Chaoborus* larvae to calculate a length-weight regression to estimate biomass. In the study lakes, Paul, Peter, Tuesday, East and West Long, there are three species: *C. punctipennis, C. flavicans,* and *C. trivittatus* that make up the chaoborid populations. All were used in the following calculations. The following sampling and measuring process was performed at 4 different times throughout the summer to obtain all of the instars needed.

SAMPLE COLLECTION:

1. Horizontal tows were taken in Paul, Peter and Tuesday Lakes after dusk with a 147 µm mesh Nitex net. The tows were pooled into 2 L jugs until ~200 larvae were collected per jug from each lake, to try to obtain as many different instars and species as possible.

2. The jugs were placed in an incubator set at ambient lake temperature overnight.

MEASURING AND WEIGHING:

1. ~1/2 of a jug was poured into an enamel pan and ~0.5 L of club soda was added to anesthetize the animals.

2. The larvae were then hand-picked and separated into instars.

3. Using a dissecting scope, the larvae were identified to species and the head capsule lengths measured to determine the instar (von Ende, 1982).

4. When ~50 larvae per species, per instar were identified, 10-15 animals were randomly chosen and length and width measurements taken. All 50 animals were then placed in a tared aluminum weighing boat and dried at 100 °C for 48 h (Fedorenko and Swift, 1972). The individual weights of each instar of each species sampled were then determined. We were able to obtain sufficient numbers of larvae for 3rd and 4th instars of *C. flavicans* and *C. punctipennis*, and 4th instar of *C. trivittatus*.

CALCULATIONS:

1. The volume of each instar for each species sampled was calculated using mean body lengths and widths, assuming the body shape of a cylinder.

2. The logs of the volumes were regressed against the logs of the individual weights. Using all species and all instars sampled, we obtained the following regression:

$\log(\text{biom}) = 1.189 \log(\text{vol}) - 8.644 \quad (r^2 = .859)$

Where:

$\text{vol} = \pi * (W/2)^2 * L$
W = width
L = length

REFERENCES:

Fedorenko, Alice Y. and Michael C. Swift. 1972. Comparative biology of *Chaoborus americanus* and *Chaoborus trivittatus* in Eunice Lake, British Columbia. Limnol. Oceanog. 17:721-730.

Roth, J.C. 1967. Notes on *Chaoborus* species from the Douglas region, Michigan, with a key to their larvae (Diptera: Chaoboridae). Pap. Mich. Acad. Sce. 52:63-68.

von Ende, Carl N. 1982. Phenology of four *Chaoborus* species. Env. Ent. 2:9-16.

(fishtech.doc)

FISH SAMPLING TECHNIQUES

The techniques used to sample the fish and estimate the population density in the cascade lakes, vary according to the species and life-stage of interest. The following techniques for the different fish populations have been used from 1984 to present, except when noted.

1. Minnows (Dace, Mudminnows, Sticklebacks and assorted small cyprinids) and Bass (young-of-year):

   The gear most used to sample these small-bodied species is the minnow trap. The traps are commercially available under the name Gee's Minnow Trap. They are made of 1/4" galvanized hardware cloth with approximately 1" openings in either end. The only modification made to the commercial trap, is treatment with acid prior to use to remove the shine of the new trap. Shiny traps have been found to be less effective. Twenty-four minnow traps are set in the littoral zone and 18 in the pelagic zone of each lake. The littoral traps are set from stakes approximately uniformly spaced around the lake at depths of .5 m and on the bottom alternately. The pelagic traps are set at 1 and 3 m across the deepest portion of the lake on a single transect with 9 stations (one 1 m and one 3 m trap at each station). The traps are set bi-weekly for 24 hr. All fish are counted and identified to species. The traps are normally set unbaited. Bait (bread) is used when large numbers of minnows must be caught, such as for a Delury estimate (Ricker, 1975).

   A subsample of approximately 400 individuals is measured. These 400 fish are anesthetized in small batches with MS-222. The anesthetized fish are placed on a clipboard covered with a sheet of transparency plastic. The nose of the minnow is placed against a retaining board and a hole is punched in the plastic at the end of the minnow's tail. The distance from the edge of the plastic to hole is measured back at the lab to obtain total length in millimeters.

   Both mark-recapture and Delury population estimation are used to estimate the abundance of minnows. With mark-recapture, a large subsample, >1000 individuals, from a minnow-trap capture are finclipped and released. Minnow traps are then reset after waiting at least 1 but not more than 7 days. The population is then estimated using the ratio of marked to unmarked fish caught in the second set. The Delury estimate is done by depleting the population using repeated minnow trapping and recording the catch and cumulative catch. The minnows are held in floating net boxes at densities of 1000-2000 per cubic meter until at least four trap sets have been made. The population is estimated by the intercept of the regression between catch and cumulative catch (the level of cumulative catch where catch equals zero). The Delury and mark-recapture estimates provides an independent population estimate to correlate the catch-per-

unit-effort (CPUE) of the regular minnow-trapping with known population densities.

2) Bass and perch (age 1+ and older):

The larger fishes are sampled for population estimation twice each year, once in mid-May and once in mid-August. The primary technique used is night electrofishing with mark-recapture population estimation.

A 16 foot Cofelt electrofishing boat, with dual booms (3 4-ft electrodes per boom), is used. Electroshocking is done largely perpendicular to shore, shocking from approximately 3 meters water depth to the shore. DC current is used to minimize damage to the fish. 600 volts provides adequate current, 2-6 amps, to stun the fish.

All fish are placed in a live-well on board the shock boat and are taken to a shore station for processing. On shore, the first 25 fish of each species are stomach pumped for gut analysis. Scales are also taken from a subsample of the fish for age analysis. All fish are identified to species, counted, measured (total length in millimeters), and weighed with either a Pesola spring-scale or an Ohaus electronic pan balance. If the spring-scale is used then the fish is simply held by the lip with the clip on the scale; if the electronic scale is used the fish is wrapped in a wet cloth to restrain the fish and the fish and cloth are weighed together. The weight of the cloth is removed by taring the scale with the cloth prior to weighing the fish.

If a mark/recapture estimation of the population is to be done, all fish sampled on the first night of electrofishing are marked. The fish are tagged with individually numbered anchor tags (Wydoski and Emery, 1983) if the fish is greater than 150mm total length and has not been previously tagged. If the fish is smaller than 150mm, the dorsal lobe of the caudal fin is clipped. The marked fish are then placed in a holding net until the first sampling is complete. The fish are released at the end of the first night of electrofishing. To increase the power of the mark-recapture technique, the number of marked fish is increased by angling and marking fish on the day prior to electroshocking at night for recapture. The population is sampled again the following night to estimate the ratio of marked to unmarked fish. For the Delury estimation, fish are removed from the lake using several days of sampling effort using both angling and electroshocking.

3) Scale Samples:

Scale samples are taken at least once a year, from at least 50 randomly selected fish of each species. Large fish are usually sampled for scales when they are caught. At least 5 scales are taken from each fish from the area below the origin of the dorsal fin and above the lateral line. Scales are permanently mounted on a plastic slide later for aging and individual growth determination (Summerfelt and Hall, 1987).

4) Larval Perch (and other pelagic larvae), 1989-present:

Two techniques are used to sample pelagic fish larvae: purse seining, and sonar.

a. Purse seining is a method of enclosing a volume of water in the pelagic zone with a net and filtering that water to obtain the larval fish. The net design and technique are described in Evans and Johannes (1988). The net used in the Cascade project is 33 meters long and 6 meters deep, made of polyester net material with 1.6mm openings and dyed green.

b. Sonar is used in conjunction with purse seining to obtain the size-frequency, species composition, and spatial distribution of icthyoplankton. The Cascade project uses both 70 and 200 khz sonar. The transducer is towed approximately 20cm under the surface. The technique is outlined in Thorne (1983). The HADAS acoustics processing hardware and software is used to analyze the recorded signal (see Rudstam, 1988, for a description of the analysis).

STATISTICAL POPULATION ESTIMATION

The two techniques used in the Cascade project to estimate population density are the modified Peterson mark-recapture and Delury estimations (Ricker 1975).

a. Mark-recapture is used for populations that are not being intentionally depleted in a lake such as the bass population in Paul Lake.

b. Delury estimates are used when a population is being removed from a lake such as for the bass in Peter Lake in the fall of 1989 or for minnows which are easily handled.

REFERENCES:

Evans, D.O. and P.R. Johannes. 1988. A bridle-less trawl and fine-mesh purse seine for sampling pelagic coregonine larvae with observations of the spatial distribution and abundance. Ontario Fish. Tech. Rep. no 27:1-19.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Department of the Environment, Fisheries and Marine Service, Fisheries Research Board of Canada Bulletin 191, Ottawa, Canada.

Rudstam, Lars G. 1988. Patterns of zooplanktivory in a coastal area of the northern Baltic proper. Doctoral thesis at the University of Stockholm.

Summerfelt, R.C. and G.E. Hall. 1987. Age and Growth of Fish. Iowa State University Press, Ames. Iowa.

Thorne, R.E. 1983. Hydroacoustics. pp. 239-260. In: L.A. Nielsen and D.L. Johnson ed. Fisheries Techniques. American Fisheries Society, Bethesda, Maryland.

Wydoski, R. and L. Emery. 1983. Tagging and Marking. pp. 215-237. In: L.A. Nielsen and D.L. Johnson ed. Fisheries Techniques. American Fisheries Society, Bethesda, Maryland.

(diet.doc)

FISH DIET ANALYSIS PROCEDURES

Fish that are 1+ and older are sampled biweekly from mid-May to late August for diet analysis, using angling as the capture method (Hodgson and Cochran, 1988). Fish are processed in less than an hour (usually much less) after capture in the following manner:

1. Between capture and release, specimens are contained in a live-well, except for rainbow trout, which are processed at the time of capture to reduce thermal related mortality. All fish are processed on shore and released at that point.

2. All gut samples are obtained from live fish using a stomach flushing technique (for the basic design of the stomach probe see Seaburg, 1957). The diameter of the afferent probe varies with the species and/or age: small for rainbow trout; 6 - 15 mm for largemouth and smallmouth bass. Water flow and pressure is maintained with a modified backpack, pesticide sprayer (Jetpak Knapsack sprayer, Ben Meadows Co., Atlanta, Georgia).

3. The gut samples are concentrated into a zooplankton cup strainer. The mesh size can be adjusted to accommodate different prey sizes (small *Daphnia* and *Polyphemus* are the smallest prey species sampled).

4. The samples are transferred to small glass vials, labeled and preserved in 95% ethanol. All gut samples are identified to nearest functional taxa (i.e. cladocerans to species; dipterans to the family - Chironomidae or to the genus - *Chaoborus*; other invertebrates to order; and fish to species).

5. For each of the diet items, the following is determined:

a. Percent number of the total sample (percent number of diet item "a" in gut sample "A", where sample A = all individuals of the sample period).
b. Percent weight of the total (same as percent number).
c. Frequency of occurrence (the number of individuals in sample "A" that contain prey item "a").

These estimates are then used in the calculation of an Index of Relative Importance. See Hodgson and Kitchell (1987) for details, as well as for the prey category weight determinations.

6. All diet data are stored on mainframe files at St. Norbert College, DePere, Wisconsin. Raw data are stored under a coding for sample period (there are 287 recorded sample periods between 1980 and 1989 - there are none for 1982). In addition, diet records of specific prey, along with other information (length, weight, catching methods and aging) for over 5,000 individual fish are found in the Cascade piscivory data files (see **Appendix V** for the input variables). The files can be found in ASCII and/or dBase III+ for the IBM, and Panorama and/or "text" formats for the MAC.

REFERENCES:

Hodgson, J.R. and P.A. Cochran. 1988. The effect of sampling methodology on diet analysis in largemouth bass (*Micropterus salmoides*). Verh. int. Ver. Limnol. 23:1670-1675.

Hodgson, J.R. and J.F. Kitchell. 1987. Opportunistic foraging by largemouth bass (*Micropterus salmoides*). Am. Mid. Natl 118:323-335.

Seaburg, K.G. 1957. A stomach sampler for live fish. Progressive Fish-Culturist. 19:137-139.

(fertmeth.doc)

ORDERING FERTILIZER AND SUPPLIES

Methods:

I. Calculate approximate amount of N and P needed for the year by first deciding on the rate of P to be applied and the N:P ratio.

A. Use the decided rate of P to be applied (mgP/m^2d) to each lake to determine total grams of P needed for each lake: Total P(g) $= \frac{P_{rate} \bullet A_L \bullet d}{1000\ mg/g}$

where P_{rate} = rate of P application (mgP/m^2d) for a particular lake

A_L = area of the lake

d = total number of days of fertilizer application

Add the amount from each lake to get a total for all lakes.

Divide this total by the estimated concentration of 85% H_3PO_4 (see table below) to determine the liters of H_3PO_4 needed using the formula:

Total H_3PO_4 (L) = $P_{tot} / [P]_{H3PO4}$

Where: P_{tot} = total grams of P needed for summer

$[P]_{H3PO4}$ = estimated concentration of 85% H_3PO_4 (g/L)

B. Use the N:P ratio set for that year and the liters of P needed to determine how much 28% N liquid fertilizer to purchase for the summer. Rearrange the equation given in **Mixing Fertilizer** to find V_{fert} and this will give the amount required for the summer. Use the table below to estimate 28% N liquid fertilizer concentrations.

	1995	1996	1997
85% H_3PO_4	418.4 gP/L	510.7 gP/L	441.37 gP/L
28% N liquid fert.	258 gN/L	277,299, 308 gN/L	These number would not be typical of actual N fertilizer concentrations because of extra that was not used in 1996.
N fert after H_3PO_4 added	13 gP/L	11.5 gP/L (average of 3 tanks)	13.5, 11,8 g P/L

II. Order fertilizer and supplies

A. H_3PO_4 comes in 15 gallon containers that weigh 220 lbs.

-containers weigh 20 lbs. + H_3PO_4 weighs 200 lbs. = 220 lbs.

-order from: Van Waters and Rogers, Inc.
1707 S. 101st St.
Milwaukee, WI 63214-0545
(414) 259-9301

B. 28 % N liquid fertilizer must be picked up in large tanks for hauling up to UNDERC.

-order from: Rib River Valley Co-op
409 Pine St.
Marathon City, WI 54448
(715) 443-2677

C. Fertilizer supplies (tanks, hoses, etc.)

-order from: Ag Systems, Inc.
6554 Hwy. 51
DeForest, WI 53532
(608) 846-9064

D. Reserve flatbed truck for hauling tanks.

-order from: Physical Sciences Lab
3725 Schneider Dr.
Stoughton, WI 53589
(608) 877-2200

MIXING FERTILIZER

Materials

Sample Jars - 125 mL wide mouth Nalgene bottles
28% N liquid fertilizer
85% H_3PO_4
4L graduated cylinder
Chemical pump

Method

I. Determine TP and TN of H_3PO_4 and liquid N fertilizer.

A. Sample collection and preparation.

1. Take samples from each tank of H_3PO_4 and liquid N fertilizer.
2. Dilute liquid N fertilizer sample 10^5 with 4 replicates.

Take a 1 mL aliquot from the sample and dilute it with water in a 100 mL volumetric flask. Invert several times. Take a 1 mL from the 10^2 solution and dilute it with water in a 1000 ml volumetric flask.

3. Dilute H_3PO_4 sample 10^7 with 3 replicates

Take a 1 mL aliquot from the sample and dilute with water in a 1000 ml volumetric flask. Invert several times. Take a 0.l mL aliquot of the 10^3 dilution and dilute with water in a 1000 mL volumetric flask.

B. Using the autoanalyser determine TP of all replicates, and TN for liquid N fertilizer only. See method for TP and TN analysis of lake water

II. Determine volume of liquid N fertilizer in tank using: $V=\pi(C/2\pi)^2h$

Where C = circumference of tank, and h = height of liquid N fertilizer in tank.

III. Add H_3PO_4 to liquid N fertilizer.

A. Determine amount to be added using the following formula:

$$\text{Vol. } H_3PO_4 \text{ to add, (L)} = \frac{\left[\frac{([N]_{fert}) \bullet (V_{fert})}{\text{N:P ratio}}\right] - \left[([P]_{fert}) \bullet (V_{fert})\right]}{[P]_{H3PO4}}$$

Where: $[N]_{fert}$ = 28% N liquid fertilizer N concentration (gN/L)
V_{fert} = Total volume of 28% N liquid fertilizer in tank (L)
$[P]_{fert}$ = 28% N liquid fertilizer P concentration (gP/L)
$[P]_{H3PO4}$ = H_3PO_4, P concentration (gP/L)
N:P ratio = this is predetermined, usually around 20 (gN/gP)

B. *** Important: Wear protective clothing when transferring H_3PO_4 to liquid N fertilizer. Use the chemical pump and 4L graduated cylinder to add H_3PO_4 to the liquid N fertilizer. Stir to mix. An old oar is useful for this job.

IV. After at least 1 day (to allow for proper dilution) mix and resample liquid N fertilizer, dilute 10^5 with 4 replicates, and analyze for final TP concentration.

FERTILIZER ADDITION

(This procedure is usually done on Friday. This allows for maximum dilution before the next sampling period.)

Materials

mixed liquid N fertilizer and H_3PO_4 (see **Mixing Fertilizer** instructions)
50 L carboys - 4 foot hose attached to spigot
4 L graduated cylinder
rubber rain gear, gloves, boots
eye protection
designated fertilizer boat
electric trolling motor and charged battery

Methods

*** IMPORTANT*** Where protective clothing whenever working with fertilizer.

1. Determine amount of fertilizer to be applied to each lake for that week. Use the following equation:

$$\text{Vol (L)} = \frac{(P_{add}) \bullet (A_L) \bullet (\text{days})}{([P]_{fert}) \bullet (1000\text{mgP}/1\text{gP})}$$

Where: P_{add} = determined rate of P addition (mgP/m^2d)
A_L = Surface area of lake (m^2)
days = number of days between fertilizer additions, usually 7 (d)
$[P]_{fert}$ = 28% N liquid fertilizer P concentration after H_3PO_4 has been mixed in (gP/L)

2. Stir liquid N fertilizer tank with old oar or wood stick. BE CAREFUL of fumes when opening lid on tank and also while stirring.

3. Fill carboys with predetermined amount of fertilizer.

4. Take carboys to lake. Do not spill carboys in state vehicles. It is preferable to use a vehicle that is not involved in regular limnological sampling to avoid possible contamination of lake chemistry samples. If you must use a limnological vehicle take great care to avoid possible spillage from the carboys by covering them in garbage bags or putting them in a plastic tub.
5. Load the carboys into the designated “fertilizer” boat. This boat should be used every week for the fertilizer addition **ONLY** and **SHOULD NOT** be used for sampling
6. While driving the boat around the deeper areas of the lake, slowly add fertilizer from the carboy to the lake. Try to cover as much area as possible during the addition. A well charged battery is very helpful in accomplishing this goal.
7. Rinse the insides and outsides of all carboys 2-3 times with lake water.

Appendix I:

CASCADE CORE DATA FILES
Last updated March 1998

The data files listed below were produced from raw Cascade field and laboratory data. The files below use a day-of-the-year system in which day 1 = January 1 (see last page for a calendar). These files also use a standard lake code in which L = Paul Lake, R = Peter Lake, E = East Long Lake, W = West Long Lake, C = Central Long Lake, and T = Tuesday Lake. The documentation below provides a SYSTAT input statement for each file which lists the variable names (in SYSTAT, a trailing $ denotes an alphabetic variable). These files are available on floppy disks readable by IBM-compatible (MS-DOS) microcomputers and are located on the Center for Limnology server in U:\Groups\Cascade\Final97!\. The files on the server can be accessed using FTP software after obtaining a user name and password. Note: In the following list, a few of the files are not yet available.

COND96.DAT: INPUT LAKE$ DAY ZID Z COND
Vertical conductivity profiles for 1996. LAKE$ is the lake identifier; DAY is the day of the year; ZID is the depth id (1-6 for our 6 sampling depths based on percent light transmission; 7=hypolimnion); Z is the depth (m); COND is the conductivity (μmhos). NOTE: CONDUCTIVITY HAS NOT BEEN MEASURED SINCE 1996.

THIS FILE NOT YET COMPLETED:
CSPP97.DAT: INPUT LAKE$ DAY SPP$ SPMDEN MLEN MWID INWT BIOM
The 1997 Chaoborus data. LAKE$ is the lake identifier; DAY is the day of the year; SPP$ is the species (t=trivitatus, f=flavicans, p=punctipennis, x=unidentified); SPMDEN is the species mean density (animals m^{-2}); MLEN is the mean length (μm); MWID is the mean width (μm) at the third body segment; INWT is the mean individual dry biomass (μg); BIOM is the total dry biomass ($g\ m^{-2}$).

DAYSOL97.DAT: INPUT DAY EINM2D
The irradiance data for 1997. DAY is the day of the year; EINM2D is irradiance in Einst m^{-2} day^{-1}.

EFFIC97.DAT: INPUT LAKE$ TAX$ EF87 EF88 EF89 EF90 EF91 EF92 EF93 EF94 EF95 EF96 EF97 AVG
The net efficiency data for 1987-1997. Please see the Cascade Methods Manual for an explanation of net efficiency calculations. LAKE$ is the lake identifier; TAX$ is the taxonomic group (see below); EF## is the raw net efficiency for a given year; AVG is the average raw net efficiency from 87-96.

The taxonomic groups (TAX$) are as follows:

DAPHN	Daphnia sp.
HGIB	Holopedium sp.
DIAPH	Diaphanasoma sp.
BOS	Bosmina sp.

POLY Polyphemus sp.
CHYD/ALONA Chydorus sp.and Alona sp.
CDAP Ceriodaphnia sp.
CYCLO Cyclopoid copepods
COPD Copepodites
NAUP Copepod nauplii
CALAN Calanoid copepods
ASPLG Asplanchna sp.
CCOL colonial Conochilus
POLAR Polyarthra sp.
SOFT Soft-bodied rotifers including Ascomorpha, Gastropus, Conochilus, Ploesoma, and Synchaeta spp.
HARD Hard-bodied rotifers including Filinia, Keratella, Kellicotia, Euchlanis, Lecane and Trichocerca spp.

FERT9397.DAT: INPUT YR WEEK LAKE$ BEGINDAY ZTHER VOLADD PADD NADD PAREAL NAREAL PVOL NVOL TDAY
The 1993-1997 fertilization. YR is the year; WEEK is the week number since the beginning of fertilization; LAKE$ is the lake identifier; BEGINDAY is the day number when fertilization began for given WEEK; ZTHER is the depth of thermocline (m); VOLADD is the volume of fertilizer added during WEEK (L); PADD is the phosphorus added during WEEK (g); NADD is the nitrogen added during WEEK (g); PAREAL is the phosphorus added per lake area per day using Downing's hypsometry (mg m^{-2} day^{-1}); NAREAL is the nitrogen added per lake area per day using Downing's hypsometry (mg m^{-2} day^{-1}); PVOL is the phosphorus added per epilimnetic volume per day using Downing's hypsometry (µg L^{-1} day^{-1}); NVOL is the nitrogen added per epilimnetic volume per day using Downing's hypsometry (µg L^{-1} day^{-1}); TDAY is a time definition used for graphing which defines the year and day.

INLITE97.DAT: INPUT DAY RISE START END EINS
The timing data for 1997 ^{14}C incubations. DAY is the day of the year; RISE is time of sunrise; START is the start time for the ^{14}C incubations; END is the end time for the ^{14}C incubations; EINS is the total incident light (Einst m^{-2}) at the lakes surface during the incubations.

NUT97.DAT: INPUT LAKE$ DAY ZID Z TP TN NH34 NO23 PO4
The 1997 chemistry data obtained from the Lachat auto-analyzer. LAKE$ is the lake identifier; DAY is the day of the year; ZID is the depth id (1-6 for our 6 sampling depths based on percent light transmission; 7=hypolimnion); Z is the depth (m); TP is the total phosphorus concentration (µg L^{-1}); TN is the total nitrogen concentration (µg L^{-1}); NH34 is the ammonia concentration (NH3 and NH4+, µg L^{-1}); NO23 is the nitrite plus nitrate concentration (µg L^{-1}); PO4 is the ortho-phosphate concentration (µg L^{-1}).

NUT9197.DAT: INPUT LAKE$ YR DAY ZID Z TP TN NH34 NO23 PO4 TDAY
The 1991-1997 chemistry data obtained from the Lachat auto-analyzer. The Systat variabe names are the same as NUT97.DAT with the addition of YR which is the year.

PCHEM97.DAT: INPUT LAKE$ DAY Z T DO IWAT IDECK.
The 1997 physical/chemical data for all lakes. LAKE$ is the lake identifier; DAY is the day of the year; Z is depth (m); T is the temperature (EC); DO is dissolved oxygen (mg L^{-1}); IWAT is the irradiance at depth (µEinst m^{-2} s^{-1}), and IDECK is the deck cell irradiance (µEinst m^{-2} s^{-1}).

PCHEM917.DAT: INPUT LAKE$ YR DAY Z T DO IWAT IDECK TDAY
The 1991-1997 physical/chemical data for all lakes. The Systat variable names are the same as PCHEM97.DAT with the addition of YR which is year.

PHYS97.DAT: INPUT LAKE$ DAY ZTHER EPIT Z1MGDO ZMAXDO Z1LT THERLT SECCHI RAIN STAFF
The 1997 physical data. LAKE$ is the lake identifier; DAY is the day of the year; ZTHER is the depth (m) of thermocline calculated as the half meter depth with maximum temperature change both above and below in the water column; EPIT is the epilimnetic temperature (EC); Z1MGDO is the depth (m) of 1 mg L^{-1} dissolved oxygen (note that if 1 mg L^{-1} d.o. is not reached, value is set to 100); ZMAXDO is the depth (m) of the maximum dissolved oxygen; Z1LT is the depth (m) of 1% surface irradiation; THERLT is the percent of surface irradiation at the thermocline; SECCHI is the Secchi depth (m); RAIN is the rainfall for the previous 24 hours; STAFF is the staff gauge reading.

PHYS9097.DAT: INPUT LAKE$ YR DAY ZTHER EPIT Z1MGDO ZMAXDO Z1LT THERLT SECCHI RAIN STAFF TDAY
The 1990-1997 physical data. The Systat variable names are the same as PHYS97.DAT with the addition of YR which is year.

THIS FILE NOT YET COMPLETED:
PP97.DAT: INPUT LAKE$ DAY YR CODE$ CODE CONC CLASS$ GALD VOL TOTVOL BVOL TOTBVOLThe 1997 phytoplankton data. LAKE$ is the lake identifier; DAY is the day of the year; YR is the year; CODE$ is the species code name (applicable 84-92); CODE is a numeric species identifier (see following list for 93-96), introduced in 1993 and replacing CODE; CONC is the concentration in natural units per mL (i.e. colony or cell, depending on how it occurs in a mounted sample); CLASS$ is the algal type division (C=Chlorophyta, Y=Chrysophyta, E=Euglenophyta, D=Dinoflagellates, R=Chryptophyta, B=Blue green, T=Diatom, .=Miscellaneous); GALD is the Greatest Axial Linear Dimension (µm); VOL (µm^3) is the individual volume of cells or colonies, including loricae and sheaths; TOTVOL (mm^3/L) is the total volume of the cells or colonies, including loricae and sheaths, of a particular taxa; BVOL (µm^3) is the individual biovolume of protoplasm, excluding loricae and sheaths; TOTBVOL (mm^3/L) is the total biovolume of protoplasm, excluding lorica and sheaths.

1996 SPECIES LIST
(Note: This species list may only be correct for 1996 data. For the most accurate species list for a particular year please use the one included in the report from Phycotech.)

Code	Taxa	Division
1010	Achnanthes sp.	Diatom
1013	Achnanthes minutissima	Diatom
1021	Asterionella formosa	Diatom

1071	Cyclotella sp. 1	Diatom
1115	Cymbella minuta	Diatom
1121	Dinobryon bavaricum	Chrysophyte
1122	Dinobryon bavaricum	Chrysophyte
1123	Dinobryon monads	Chrysophyte
1124	Dinobryon cylindricum	Chrysophyte
1125	Dinobryon cylindricum	Chrysophyte
1126	Dinobryon cyst	Chrysophyte
1127	Dinobryon divergens	Chrysophyte
1128	Dinobryon divergens	Chrysophyte
1129	Dinobryon sertularia	Chrysophyte
1140	Eunotia sp.	Diatom
1152	Fragilaria crotonensis	Diatom
1154	Fragilaria construens	Diatom
1160	Gomphonema sp.	Diatom
1180	Mallomonas sp.	Chrysophyte
1183	Mallomonas akrokomos	Chrysophyte
1184	Mallomonas acaroides	Chrysophyte
1185	Mallomonas caudata	Chrysophyte
1190	Melosira sp.	Diatom
1214	Navicula cryptocephala	Diatom
1223	Nitzschia perminuta	Diatom
1310	Synedra sp.	Diatom
1320	Synura sp. (colonial)	Chrysophyte
1323	Synura sp. (single)	Chrysophyte
1324	Synura spinosa	Chrysophyte
1325	Synura spinosa	Chrysophyte
1326	Synura sphagnicola	Chrysophyte
1327	Synura sphagnicola	Chrysophyte
1331	Tabellaria fenestrata	Diatom
1332	Tabellaria flocculosa	Diatom
1401	Bitrichia ochridana	Chrysophyte
1415	Dinobryon sp. 2	Chrysophyte
1418	Dinobryon sp. 4	Chrysophyte
1422	Dinobryon sp. 7	Chrysophyte
1470	Chrysococcus sp.	Chrysophyte
1481	Chrysosphaerella longispina (single)	Chrysophyte
1482	Chrysosphaerella longispina (colonial)	Chrysophyte
1490	Chrysolykos sp.	Chrysophyte
1561	Kephyrion sp. 1	Chrysophyte
1563	Kephyrion sp. 3	Chrysophyte
1564	Kephyrion sp. 4	Chrysophyte
1568	Kephyrion sp. 8	Chrysophyte
1570	Ochromonas sp.	Chrysophyte
1611	Stichogloea olivacea	Chrysophyte
1630	Uroglena sp. (single)	Chrysophyte

1631	Uroglena sp. (colonial)	Chrysophyte
1653	Cyst (Chrysophyte)	Chrysophyte
1731	Erkenia subaequiciliata	Chrysophyte
2001	Scenedesmus incrassatulus	Chlorophyte
2031	Ankistrodesmus falcatus	Chlorophyte
2035	Ankistrodesmus convolutus	Chlorophyte
2041	Botryococcus braunii	Chlorophyte
2061	Carteria platyrhyncha	Chlorophyte
2071	Characium limnetcum	Chlorophyte
2080	Chlamydomonas sp.	Chlorophyte
2082	Chlamydomonas globosa	Chlorophyte
2083	Chlamydomonas incerta	Chlorophyte
2085	Chlamydomonas platystigma	Chlorophyte
2151	Closteriopsis longissima	Chlorophyte
2162	Closterium moniliferum	Chlorophyte
2171	Coelastrum microporum	Chlorophyte
2175	Coelastrum pseudomicroporum	Chlorophyte
2180	Cosmarium sp.	Chlorophyte
2192	Crucigenia rectangularis	Chlorophyte
2211	Dictyosphaerium pulchellum	Chlorophyte
2231	Elakatothrix gelatinosa	Chlorophyte
2232	Elakatothrix viridis	Chlorophyte
2282	Gloeocystis ampla	Chlorophyte
2300	Gonium sp.	Chlorophyte
2331	Micractinium pusillum	Chlorophyte
2340	Mougeotia sp.	Chlorophyte
2363	Oocystis parva	Chlorophyte
2367	Oocystis pusilla	Chlorophyte
2462	Quadrigula lacustris	Chlorophyte
2481	Scenedesmus sp. 2	Chlorophyte
2483	Scenedesmus bijuga	Chlorophyte
2484	Scenedesmus abundans	Chlorophyte
2488	Scenedesmus denticulatus	Chlorophyte
2491	Schroederia judayi	Chlorophyte
2500	Selenastrum sp.	Chlorophyte
2504	Selenastrum gracile	Chlorophyte
2532	Staurastrum ophiura	Chlorophyte
2535	Staurastrum cingulum	Chlorophyte
2538	Staurastrum hexacerum	Chlorophyte
2554	Tetraedron minimum	Chlorophyte
2558	Tetraedron trigonum	Chlorophyte
2590	Ulothrix sp.	Chlorophyte
2600	Volvox sp.	Chlorophyte
2632	Arthrodesmus subulatus	Chlorophyte
2641	Sphaerocystis schroeteri	Chlorophyte
2681	Colonial chlorophyta - type 1	Chlorophyte
2682	Colonial chlorophyta - type 2	Chlorophyte

2683	Non-motile Chlorococcales-spher	Chlorophyte
2686	Cyst (Chlorophyte)	Chlorophyte
2687	Non-motile Chlorococcales-ovoid	Chlorophyte
2811	Tetracystis pulchra	Chlorophyte
2840	Lobomonas sp.	Chlorophyte
2851	Lagerheimia ciliata	Chlorophyte
2861	Monomastix astigmata	Chlorophyte
2871	Paulschulzia tenera	Chlorophyte
2884	Scenedesmus quadricauda	Chlorophyte
2889	Scenedesmus armatus	Chlorophyte
2892	Staurastrum paradoxum	Chlorophyte
2894	Staurastrum iotanum	Chlorophyte
2920	Xanthidium sp.	Chlorophyte
2931	Teilingia granulata	Chlorophyte
3015	Cryptomonas erosa	Cryptophyte
3018	Cryptomonas lucens	Cryptophyte
3020	Chroomonas sp.	Cryptophyte
3061	Cryptomonas ovata	Cryptophyte
3062	Cryptomonas caudata	Cryptophyte
3068	Cryptomonas cyst	Cryptophyte
4012	Anabaena flos-aquae	Cyanophyte
4051	Aphanocapsa elachista	Cyanophyte
4054	Aphanocapsa delicatissima	Cyanophyte
4062	Aphanothece nidulans	Cyanophyte
4065	Aphanothece clathrata	Cyanophyte
4082	Chroococcus limneticus	Cyanophyte
4161	Merismopedia tenuissima	Cyanophyte
4172	Oscillatoria limnetica	Cyanophyte
4174	Oscillatoria tenuis	Cyanophyte
4191	Phormidium mucicola	Cyanophyte
4242	Gomphosphaeria lacustris	Cyanophyte
4285	Non-motile blue-greens (>2 UM)	Cyanophyte
4286	Non-motile blue-greens - ovoid	Cyanophyte
4304	Lyngbya sp. 3	Cyanophyte
4365	Oscillatoria subuliformis	Cyanophyte
4462	Pseudanabaena biceps	Cyanophyte
5022	Euglena gracilis	Euglenophyte
5023	Euglena acus	Euglenophyte
5046	Trachelomonas sp.	Euglenophyte
5047	Trachelomonas volvocina	Euglenophyte
6011	Ceratium hirundinella	Dinoflagellate
6032	Gymnodinium sp. 1	Dinoflagellate
6033	Gymnodinium sp. 2	Dinoflagellate
6034	Gymnodinium sp. 3	Dinoflagellate
6040	Peridinium sp.	Dinoflagellate
6041	Peridinium cinctum	Dinoflagellate
6044	Peridinium umbonatum	Dinoflagellate

6051	Dinoflagellate cyst	Dinoflagellate
7080	"Misc. micros, 1 flagellum"	Miscellaneous
7090	"Misc. micros, 2 flagella"	Miscellaneous
7111	Gonyostomum semen	Miscellaneous
7140	Misc. microflagellate	Miscellaneous
8011	Deasonia gigantica	Chlorophyte
8041	Monoraphidium capricornutum	Chlorophyte
8081	Cystomonas starrii	Chlorophyte
8091	Schizochlamys compacta	Chlorophyte
8304	Scenedesmus aculeolatus	Chlorophyte
8308	Scenedesmus serratus	Chlorophyte
8313	Chlamydomonas sp. 3	Chlorophyte
8351	Chloromonas pumilio	Chlorophyte
9141	Anomoeoneis vitrea	Diatom
9504	Synedra tenera	Diatom
9511	Polygoniochloris circularis	Chrysophyte

PRCS97.DAT: INPUT LAKE$ DAY ZID Z DIC PPR1 PPR2 CHLA PHEO CHLA35 PHEO35.The 1997 process data. LAKE$ is the lake identifier; DAY is the day of the year; ZID is the depth identifier (1-6); Z is the sampling depth (m); DIC is the dissolved inorganic carbon (mg C L^{-1}) determined from the gas chromatograph; PPR1-2 are primary production (mg C m^{-3} h^{-1}); APA is the alkaline phosphatase activity (nmol PO_4 min^{-1})(APA has not been performed since 1995 and is now longer a column in the data set.); CHLA is the total chlorophyll a concentration (µg L^{-1}); PHEO is the total phaeopigment concentration (µg L^{-1}); CHLA35 is the edible (<35 µm) chlorophyll a concentration (µg L^{-1}); PHEO35 is the edible (<35 µm) phaeopigment concentration (µg L^{-1}).

TRAP97.DAT: INPUT LAKE$ BEGIN END TRAP TPTR TPHY TPRAT TNTR TNHY TNRAT
The 1997 sediment trap data. LAKE$ is the lake identifier; BEGIN is the day of the trap deployment; END is the day of the trap retrieval; TRAP is the trap identifier (1 or 2); TPTR is the total phosphorus (µg L^{-1}) in the sediment trap; TPHY is the total phosphorus (µg L^{-1}) in the hypolimnion on the date the traps were pulled (or nearest date); TPRAT is the phosphorus sedimentation rate (µg m^{-2} d^{-1}); TNTR is the total nitrogen (µg L^{-1}) in the sediment trap; TNHY is the total nitrogen (µg L^{-1}) in the hypolimnion on the date the traps were pulled (or nearest date); TNRAT is the nitrogen sedimentation rate (µg m^{-2} d^{-1}). Trap depths are 12 m in all lakes but Paul, where trap depth is 9 m (Note: In past years, TPHY and TNHY were values in the middle of the sedimentation period as opposed to the end of the sedimentation period as in 1996.)

ZOOP9097.DAT: INPUT LAKE$ YR DAY TAX$ GRP$ EFF NHAUL DENS LEN INWT BIOM TDAY
The 1990-1997 zooplankton data. The Systat variable names are the same as ZOOP97.DAT with the addition of YR which is year. NOTE: ROTIFERS WERE NOT COUNTED IN 1997!

ZOOP97.DAT: INPUT LAKE$ DAY TAX$ GRP$ EFF NHAUL DENS LEN INWT BIOM

The 1997 zooplankton data. LAKE$ is the lake identifier; DAY is the day of the year; TAX$ is the taxonomic code (see below); GRP$ is an arbitrary grouping code (CLAD = cladoceran; ROT = rotifer; PROT = predaceous rotifer; CCOP = carnivorous copepod; OCOP = omnivorous copepod); EFF is the net efficiency; NHAUL is the number of animals in one haul; DENS is the density (animals L^{-1}); LEN is the mean length (mm); INWT is mean individual dry biomass (µg); BIOM is the total dry biomass (µg L^{-1}). Net haul depths are: Paul Lake = 8 m, Peter Lake = 12 m, East Long Lake = 12 m, and West Long Lake = 12 m. NOTE: ROTIFERS WERE NOT COUNTED IN 1997!

The taxonomic code (TAX$) is as follows:

DPUL	Daphnia pulex
DROS	Daphnia rosea
DJUV	Juvenile Daphnia sp. (first documented in 1997)
DDUB	Daphnia dubia
DPRV	Daphnia parvula
POLY	Polyphemus sp.
HGIB	Holopedium gibberum
BOS	Bosmina longirostris
CDAP	Ceriodaphnia sp.
ALON	Alona sp.
CHYD	Chydorus sp.
DIAPH	Diaphanosoma birgei
SCRYS	Sida crystallina
MESO	Mesocyclops edax
ORTH	Orthocyclops modestus
DTHO	Diacyclops thomasi
ACAN	Acanthocyclops sp. (first documented in 1996)
CYCVR	Cyclops varicans rubellus
TROP	Tropocyclops prasinus mexicanus
DIAPT	Diaptomus sp.
EPSH	Epischura lacustris
COPD	Copepodite
NAUP	Nauplii
PVULG	Polyarthra vulgaris
PMAJ	Polyarthra major
GSTYL	Gastropus stylifer
GHYPT	Gastropus hyptopus
CDOS	Conochiloides dossuarius
AEUC	Ascomorpha ecaudis
SYNCH	Synchaeta sp.
ASPLG	Asplanchna sp.
KCOCH	Keratella cochlearis
KTEST	Keratella testudo
KTAU	Keratella taurocephala
KLONG	Kellicotia longispina
KBOST	Kellicotia bostoniensis
FIL	Filinia sp.

TMULT	Trichocerca multicrinis
TCYL	Trichocerca cylindrica
PLOES	Ploesoma sp.
PHUD	Ploesoma hudsoni
CCOL	colonial Conochilus sp.
BANG	Brachionus angularis
LEC	Lecane sp.
EUCH	Euchlanis sp.

APPENDIX II: DATASET DOCUMENTATION: (prgmdoc2.doc)

HOW TO DEAL WITH DATA DURING THE FIELD SEASON & HOW TO CREATE ASCII FILES ONCE IT'S OVER

Variable	Excel Filename	Columns in Excel & .CSV Files	Command Files (for Systat DATA module)
AER	AER9?.XLS	lake$ day layer$ time0 +n1 +n2 +n3, con1 con2 con3	CALCAER.CMD
APA	APA9?.XLS	lake$ day zid z scale repl# time0 time10 time20 time30 time40	CALCAPA.CMD
Chaoborus	CSPP9?.XLS CDEN9?.XLS	lake$ day spp$ head body wid scope (one line per Chaoborus measured) lake$ day #inTow1 #inTow2 #inTow3 avgdens (one line per sample) (avgdens = mean / eff; eff=0.575)	1. Run MKCHAOB1.CMD 2. Run FIXCHAOB.WPM in WP51 3. Run MKCHAOB2.CMD
Chlorophyll	CHL9?.XLS	lake$ day zid z totvol totfb totfa totscale filtvol filtfb filtfa filscale fluor$	CALCCHL.CMD
Conductivity	COND9?.XLS	lake$ day depth cond	-- none --
Pyroheliometer light data	DAYSOL9?.XLS	day einm2d	-- none --
Pyro light data for PPRs	INLITE9?.XLS	day rise start end einm2d	-- none --
DIC	DIC9?.XLS	lake$ day zid z blank1 blank2 std1 std2 std3 std4 avgcnts	CALCDIC.CMD
Diels	DIEL9?.XLS	lake$ day time$(=D or N) DpulA DpulB DpulC DrosA DrosB DrosC BosA BosB BosC Copavg	-- none --
Gran Alkalinity	GRAN9?.XLS	lake$ day layer$ pH voladded inittemp	CALCGRAN.CMD

Variable	Excel Filename	Columns in Excel & .CSV Files	Command Files (for Systat DATA module)
Temp,DO, and light data from routines data sheet	PCHEM9?.XLS	lake$ day z temp DO iwat ideck	-- none --
Physical data from routines data sheet	PHYS9?.XLS	lake$ day zther epit z1mgdo zmaxdo z1lt therlt secchi rain staff	-- none --
PPR (measured)	PPR9?.XLS	lake$ day zid z tot1 tot2 tot3 lite1 lite2 dark hours	CALCPPR.CMD
Daily integrated PPR (calculated)	PRCS9?.XLS PCHEM9?.XLS INLITE9?.XLS IRREX9?.SYS DAYSOL9?.XLS	most of these are described elsewhere on this table. IRREX9?.SYS is the same as INLITE9?.XLS, with an added column for irradiance rate, "irrate," where irrate = einm2/(end-start)	See Appendix III for involved description of calculation process.
Process data	PRCS9?.XLS	lake$ day zid z dic ppr1 ppr2 apa chla pheo chla35 pheo35	-- none --
Zooplankton (net efficiencies)	EFF9?.XLS	lake$ tax$ ef87 ef88 ef89 ef90 ef91 ef92 ef93 ...ef9?	-- none --
Zooplankton (templates for each lake)	ZOOP?9?.XLS	lake$ day tax$ nin2haul inwt len eff se.len lengths	-- none --
Zooplankton (summary file)	ZP9?.XLS	lake$ day tax$ nin2hauls inwt len eff	ZOOPCONV.CMD (calls 3 subprograms) a. FIXMISS.CMD - fills in missing lengths b. ADDROTLN.CMD - adds rotifer lengths c. CALCINWT.CMD - calculates biomass
Nutrients	NUT9?.XLS	lake$ day zid z TP TN NH3 NO23 PO4	-- none --
Sediment traps	TRAP9?.XLS	lake$ begin end trap tptr tphy tprat tntr tnhy tnrat	-- none --

(pprprog.doc)

APPENDIX III: CASCADE PRIMARY PRODUCTIVITY COMPUTER PROGRAMS

This is the documentation for the CASCADE primary productivity Computer Programs. The programs can be used to calculate PPR for any year.

DATA SETS: To calculate PPR, you need the following data sets:

PRCS9? (systat)
PCHEM9? (systat)
IRREX9? (systat)
DAYSOL9? (ascii)

SEQUENCE:

1. In Systat data module, run PPRSET9?.CMD. This program calculates input data sets for curve fits to predict specific primary production rate from physical-chemical variables.

2. If you wish to perform regressions on multiple years of data, concatenate results of PPRSET runs for all years of interest.

3. In Systat MGLH module, run PIFIT.CMD to fit the regressions. Generally you will have to examine significance tests, the error MS, and residuals to decide which model is best for each lake. This will probably require several runs of the program, modifying the predictor variables in each run.

4. In Systat data module, run FILE9?.CMD to create an input data set for the PPRCAL3 program.

5. Use a text editor to incorporate your regression equations into PPRCAL3.C. The equations are clearly marked by comments in the program, and are found near lines 279-298.

6. Recompile PPRCAL3 using Turbo C 2.0 or a later, compatible version of that compiler.

7. Run PPRCAL3, which will calculate daily PPR and create DAPPR_ files for each lake.

APPENDIX IV: LACHAT SOFTWARE COMMANDS

The following is an outline of necessary commands used to run the Lachat autoanalyzer software in order to perform nutrient analyses.

MAIN MENU

A. METHODS >

METHOD DEFINITION > **.....Choose Method**
Channels > Select > Standards > Concentrations **....Enter standard concentrations**
> Evaluations > Calibration > Boundary **.....Check Boundary settings**
Esc **.....Exit and Save**

ANALYSIS SELECT AND DOWNLOAD **.....Download Method**

SUBMIT>
Calibrate Now **.....Calibrate machine**

B. RESULTS / APPROVAL >

Calibration Graphs and Stats > Calib. Graphs **.....View calibration graphs**
Approval **.....Approve calibration**

NOTE Once the calibration is approved, do NOT download again or else the calibration will be lost.

C. SAMPLES > Tray Definition and Submit .>

Edit > Identification **.....Type in sample ID's**
File > Write Template (Public) **.....Save sample ID's**
File > Read Template (Public) **.....Recall sample ID's (if lost)**
Submit > Submit Current Tray **.....Submit tray with listed ID's**

NOTE F8, F7, and F6 keys can be used to save time in typing in sample ID's. Up to 3 separate trays can be submitted concurrently.

D. RESULTS / APPROVAL > View Calibration and Sample Reports >

Tray Selection > Sample Trays **.....Choose correct sample tray**
Reports > Choose a Report Definition **.....Choose chord of best calibration**
> Browse Report
> Save Report **.....Save Report to Disc**

Appendix V: Zooplankton Length Weight Regression and Rotifer Lengths

Length Weight Regressions

```
REM  CALCINWT.CMD
```

```
REM  KLC created 19 January 1994
```

```
REM  last modified 25 Feb, 1997 (added ACAN)
```

```
REM  formulas for zooplankton biomass based on length

REM  These have been checked with Downing & Rigler (1984) & Soranno (1990)
REM  as well as with zooplankton notes for 1984-1989 (e.g. Soranno 1989)

REM  A number of formulas have been changed back to 1989 versions, since
REM  alterations between 1989 and 1994 were NOT documented sufficiently
REM  to merit retention.
REM  Modified for 97 work on 1-21-98 by DLB; added DJUV.

REM -----------------------------------------------------------------------
REM                 CLADOCERANS
REM -----------------------------------------------------------------------

if tax$='DPUL' then let inwt=EXP(1.9445+(2.72*(log(len))))
      rem source is Daphnia pulex in O'Brien and deNoyelles 1974 (D&R)
if tax$='DDUB' then let inwt=EXP(1.9445+(2.72*(log(len))))
if tax$='DROS' then let inwt=EXP(1.9445+(2.72*(log(len))))
if tax$='DPRV' then let inwt=EXP(1.9445+(2.72*(log(len))))
if tax$='CDAP' then let inwt=EXP(1.9445+(2.72*(log(len))))
if tax$='DJUV' then let inwt=EXP(1.9445+(2.72*(log(len))))

if tax$='BOS' then let inwt=EXP(2.7116+(2.5294*(log(len))))
      rem source is Bosmina longirostris in Bottrell et al. 1976 (D&R)
if tax$='CHYD' then let inwt=EXP(2.7116+(2.5294*(log(len))))
if tax$='ALON' then let inwt=EXP(2.7116+(2.5294*(log(len))))
if tax$='ALONA' then let inwt=EXP(2.7116+(2.5294*(log(len))))
      rem use same as Bosmina (Soranno, 1989)
      rem alternative: inwt=EXP(4.543+(3.636*(Log(len))))
      rem whose source is Chydorus sphaericus in Rosen 1981 (D&R)
      rem problem is, the Rosen formula gives 30-40 ug/l Alona

if tax$='DIAPH' then let inwt=EXP(1.6242+(3.0468*(log(len))))
      rem source is Diaphanosoma brachyrum in Bottrell et al. 1976 (D&R)

if tax$='SCRYS' then let inwt=EXP(2.0539+(2.189*(log(len))))
      rem source is Sida crystallina in Bottrell et al. 1976 (D&R)

if tax$='HGIB' then let inwt=9.86*((len)^2.1)
if tax$='POLY' then let inwt=9.86*((len)^2.1)
      rem source is Peters and Downing (1984) general zooplankton regression
      rem did NOT use formulas from Downing and Rigler (1984)
REM -----------------------------------------------------------------------
REM                 CALANOID COPEPODS
REM -----------------------------------------------------------------------

if tax$='DIAPT' then let inwt=EXP(1.2431+(2.2634*(log(len))))
if tax$='DOREG' then let inwt=EXP(1.2431+(2.2634*(log(len))))
if tax$='LEPTO' then let inwt=EXP(1.2431+(2.2634*(log(len))))
```

```
if tax$='SDIAP' then let inwt=EXP(1.2431+(2.2634*(log(len))))
      rem source is Diaptomus gracilis in Bottrell et al. 1976 (D&R)

if tax$='EPSH' then let inwt=EXP(1.2431+(2.2634*(log(len))))
      rem use same as Diaptomus (Soranno 1989)

REM ------------------------------------------------------------------------
REM                  CYCLOPOID COPEPODS
REM ------------------------------------------------------------------------

if tax$='MESO' then let inwt=EXP(1.6602+(3.968*(log(len))))
      rem source is Mesocyclops edax in Rosen 1981 (D&R)

if tax$='ACAN' then let inwt=EXP(2.0577+(2.553*(log(len))))
if tax$='ORTH' then let inwt=EXP(2.0577+(2.553*(log(len))))
if tax$='DTHO' then let inwt=EXP(2.0577+(2.553*(log(len))))
if tax$='CYCVR' then let inwt=EXP(2.0577+(2.553*(log(len))))
if tax$='DT-CYV' then let inwt=EXP(2.0577+(2.553*(log(len))))
if tax$='TROP' then let inwt=EXP(2.0577+(2.553*(log(len))))
      rem source is Cyclops vicinus in Botrell et al. 1976 (D&R)

REM ------------------------------------------------------------------------
REM                  IMMATURE COPEPODS
REM ------------------------------------------------------------------------

if tax$='COPD' then let inwt=EXP(2.0577+(2.553*(log(len))))
      rem source is Cyclops vicinus in Botrell et al. 1976 (D&R)

if tax$='NAUP' then let inwt=EXP(0.6977+(0.469*(log(len))))
      rem source is copepod nauplii in Rosen 1981 (D&R)
```

```
REM ------------------------------------------------------------------
```

```
REM        ROTIFERS (all in D&R 1984, p.247-249, column 3)
REM ---------------------------------------------------------------------

if tax$='AEUC' then let inwt=100*0.12*((len)^3)
      rem Ascomorpha
if tax$='ASPLG' then let inwt=100*0.23*((len)^3)
      rem Asplanchna
if tax$='BANG' then let inwt=100*0.12*((len)^3)
      rem Brachionus
if tax$='EUCH' then let inwt=100*0.1*((len)^3)
      rem Euchlanis
if tax$='LEC' then let inwt=100*0.1*((len)^3)
      rem Lecane (assumed same as Euchlanis)
if tax$='FIL' then let inwt=100*0.13*((len)^3)
if tax$='FLONG' then let inwt=100*0.13*((len)^3)
if tax$='FTERM' then let inwt=100*0.13*((len)^3)
      rem Filinia
if tax$='GHYPT' then let inwt=100*0.2*((len)^3)
if tax$='GSTYL' then let inwt=100*0.2*((len)^3)
      rem Gastropus  (same as historical)
if tax$='CDOS' then let inwt=100*0.2*((len)^3)
      rem Conochiloides (assumed same as Gastropus, Soranno 1989)
if tax$='SYNCH' then let inwt=100*0.12*((len)^3)
      rem Synchaeta (assumed same as Gastropus, Soranno 1989)
if tax$='KLONG' then let inwt=100*0.03*((len)^3)
if tax$='KBOST' then let inwt=100*0.03*((len)^3)
      rem Kellicotia
if tax$='KCOCH' then let inwt=100*0.02*((len)^3)
if tax$='KTEST' then let inwt=100*0.02*((len)^3)
if tax$='KTAU' then let inwt=100*0.02*((len)^3)
      rem Keratella
if tax$='PHUD' then let inwt=100*0.15*((len)^3)
if tax$='PLOES' then let inwt=100*0.15*((len)^3)
      rem Ploesoma (average of P.hudsonii and P. triacanthum)
if tax$='PVULG' then let inwt=100*0.28*((len)^3)
if tax$='PMAJ' then let inwt=100*0.28*((len)^3)
      rem Polyarthra (same as historical)

REM species where we should measure length and width : assign average inwt
if tax$='CCOL' then let inwt=2.910
      rem Conochilus colonies; 1984-1990 long-term mean
if tax$='TCYL' then let inwt=0.124
if (tax$='TMULT' or tax$='TMUL')  then let inwt=0.068
      rem Trichocerca; 1984-1990 long-term means
```

Rotifer Lengths

```
rem addrotln.cmd
```

```
rem KLC, written by mkaddrot.cmd Jan. 19, 1994
```

```
rem  last modified Feb. 25, 1997 (only added BRACH species)
rem adapted for use with 1996 data

rem these values are primarily from 84-90 means in all lakes

 IF TAX$='CHYD' and len=. then let len=0.24
       rem * this is "ave" value in 96 (often forget to measure)
 IF TAX$='AEUC' Then let  len=0.142
 IF TAX$='ASPLG' and len=. then let  len=0.501
       rem measured in 1995; only fill in where Nick forgot
 IF TAX$='CCOL' Then let  len=0.145
 IF TAX$='BANG' and len=. then let len=0.22
       rem measured in 1996; only fill in where Nick forgot
 IF TAX$='CDOS' Then let  len=0.133
 IF TAX$='CSING' Then let  len=0.099
 IF TAX$='FLONG' Then let  len=0.145
 IF TAX$='FIL' Then let  len=0.145
       rem renamed from FLONG
 IF TAX$='GHYPT' Then let  len=0.119
 IF TAX$='GSTYL' Then let  len=0.106
 IF TAX$='KBOST' Then let  len=0.123
 IF TAX$='KCOCH' Then let  len=0.111
 IF TAX$='KLONG' Then let  len=0.137
 IF TAX$='KTAU' Then let  len=0.116
 IF TAX$='KTEST' Then let  len=0.119
 IF TAX$='PLOES' Then let  len=0.121
 IF TAX$='PMAJ' Then let  len=0.179
 IF TAX$='PVULG' Then let  len=0.128
 IF TAX$='SYNCH' Then let  len=0.186
 IF TAX$='TCYL' Then let  len=0.337
 IF TAX$='TMULT' Then let  len=0.166

rem this batch is new since 90; were measured well in 1994
IF TAX$='LEC' and len=. then let len=0.201
IF TAX$='EUCH' then let len=0.314
```

DAY OF THE YEAR CALENDAR (calend2.doc)

Date	May	June	July	August	September	October

1	121	152	182	213	244	274
2	122	153	183	214	245	275
3	123	154	184	215	246	276
4	124	155	185	216	247	277
5	125	156	186	217	248	278
6	126	157	187	218	249	279
7	127	158	188	219	250	280
8	128	159	189	220	251	281
9	129	160	190	221	252	282
10	130	161	191	222	253	283
11	131	162	192	223	254	284
12	132	163	193	224	255	285
13	133	164	194	225	256	286
14	134	165	195	226	257	287
15	135	166	196	227	258	288
16	136	167	197	228	259	289
17	137	168	198	229	260	290
18	138	169	199	230	261	291
19	139	170	200	231	262	292
20	140	171	201	232	263	293
21	141	172	202	233	264	294
22	142	173	203	234	265	295
23	143	174	204	235	266	296
24	144	175	205	236	267	297
25	145	176	206	237	268	298
26	146	177	207	238	269	299
27	147	178	208	239	270	300
28	148	179	209	240	271	301
29	149	180	210	241	272	302
30	150	181	211	242	273	303
31	151		212	243		304

*Please note that during a leap year, one day should be added to the number read off of this table.

*Also note: The variable TDAY is used when graphing several years of data on a single axis; for data since 1990, TDAY=((YR-90)*100)+(100*(DAY-130)/135)