NTL Research Protocols

NTL-LTER

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Welcome

This is the website for NTL-LTER research protocols.

Acknowledgements

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Part I

Field Protocols

The following section contains protocols taken from https://lter.limnology.wisc.edu/research/protocols

1 Benthic Macroinvertebrates

Revised: Unknown

1.0.1 Purpose:

We sample for benthic macroinvertebrates in Trout, Crystal, and Sparkling lakes using Hester-Dendy samplers. We set samplers at four to six shoreline sites in 1 meter water depth, and at the deepest part of the lake. The same sampling sites are used every year. Three Dendy samplers per site are deployed for approximately one month beginning around the second week of August. Upon collection, we preserve the contents of each sampler in ethanol, and archive the samples in the Zoology museum.

1.0.2 Sampling:

Dendies should be placed in the LTER lakes during the last week or immediately following the last week of the LTER fish sampling, and left in the lakes for approximately four weeks. Most of the dendies are set at fyke net sites so that both fish and macroinvertebrate data are collected there, as dendies can provide an indication of the species and biomass available as fish prey. Fish sampling and macrophyte sampling must be finished before dendies are set because their work disturbs the dendy sites.

Dendies were set in all the LTER lakes through 1989, and in 1992 and 1993. Only Trout, Crystal and Sparkling lakes were sampled in 1990, 1991, and 1994 to present.

1.0.2.1 Assembly

Parts needed for one dendy sampler:

- Six wide-meshed 3" x 3" vexar mesh squares
- Four narrow-meshed 3" x 3" vexar mesh squares
- Two 3" x 3" tempered hardboards
- One 'choreboy' plastic scrubbing puff
- One 5 inch long, 1/4" diameter eyebolt with nut

Construct the dendy sampler by pushing the eyebolt through the center hole of each piece with the pieces fitting tightly on the bolt so the dendy cannot become compressed. The individual pieces are layered onto the bolt in this order: hardboard, five vexar meshes alternating wide with narrow, choreboy, five vexar meshes alternating wide with narrow, hardboard, nut. Fold the choreboy into three or four layers, with the most frayed end on the inside. The eyebolt should have a zip tie loop through it. Dendies should be assembled at the lab before going out to set them.

1.0.2.2 Placement in Lakes

A "dendy set" consists of three dendy samplers from one lake site. In each set, the middle dendy is designated 'Dendy B' and the end dendies as dendies 'A' and 'C'. Dendy sets are placed in the lakes in one of three configurations.

- Attached set with anchor and subsurface float. Most shoreline sites (also called fyke net sites) are set this way. Each set consists of a 6 meter line with a minnow trap clip at each end and one in the middle. A dendy is attached to each clip, with the middle clip also being attached to a brick anchor with subsurface float. Beginning 2010, the set in Crystal lake is anchored with a dog tieout stake pushed into the sediment. This is an attempt to reduce the migration of Dendies due to camper activity.
- Unattached set with anchor and subsurface float. Shoreline sites in Sparkling Lakes are set this way. The individual dendies are not attached together. Each dendy has its own anchor, but only the middle dendy has a float. Setting the dendies as unattached sets reduces disturbance and loss of samplers to the curious public on this high use lake.
- Attached set with surface float and no anchor. Deep hole sites (also called gill net sites) and all bog sites are set this way. Dendies are connected with a 6 meter line as for the shoreline sets, but are not anchored. The three dendies sink at a more equal rate if one of them is not weighted, hopefully coming to rest apart from one another rather than clumped together. Also, an anchor may pull the dendies into the bottom sediments.

Shoreline sets are placed parallel to shore in about one meter of water. The floats are 250ml or 500ml plastic bottles partially filled with water so that they remain submerged.

Floats should be attached directly to the brick anchors rather than to the dendy or they will pull the dendy off the lake bottom. Floats should be labeled with 'Trout Lake Station' and our phone number. Sets are assembled in the boat and simply tossed over the side at the site. They should be set so that the dendies are spread out the length of the attachment line rather than clumped together. The samplers do not have to be upright.

1.0.2.3 Retrieval

- Dendy sets remain in the lakes for about four weeks. Select a calm day for retrieval as the dendies and subsurface floats are very hard to see if there are even moderate waves

on the lake.

- Two people make up the retrieval crew: a 'boat person' and a snorkeler. Anchor the boat near the middle dendy, but collect dendies A and C before dendy B. Lying on your belly on the lake bottom, quickly place a dendy in its labeled freezer container, cover with lid, and return it to the boat person. Disturb it as little as possible before getting it contained, and be careful not to drag the other dendies when returning with it to the boat.
- Due to physical limitations, the deep sites (and bog sites if sampled) are retrieved without snorkeling. Slowly pull the dendies up until they are within reach of the boat person. Place the container in the lake beneath the dendy and lift the dendy from the water with the container.
- Unclip the line from the dendy, replace the container lid with a mesh panel lid, and drain the lake water from the container. Rinse the mesh lid into the container with 95% ethanol, filling the container about half full with EtOH. Cover the container, tilting and swirling it to immerse all the invertebrates in ethanol.
- At each site, note on the field sheet whether all dendies were recovered.

Inventory Needed	TR	CR	SP	Total
dendies	21	15	15	51
brick anchors	6	0	12	18
6-meter lines	7	5	1	13
floats	7	1	5	13

1.0.3 Equipment list for fieldwork

1.0.3.1 Setting:

- dendies, lines, floats, brick anchors
- Extras of all parts
- Maps, data sheets, pencils, sharpee, string, scissors, duct tape

1.0.3.2 Retreival

- dendy containers w/ lids, big gray transport tubs
- Mesh strainer lids, 95% EtOH in gallon jugs, EtOH wash bottle
- Foam board to wrap lines on
- Maps and data sheets, pencils, sharpee, labeling tape, scissors
- Anchor for boat, snorkeling gear (plenty of weight)
- Hook to retrieve dendies set or moved into too deep water

1.0.4 Processing

- Dendies should be processed as soon as possible after collection to avoid desiccation due to evaporation of the ethanol. The freezer container lids do not fit tightly enough to allow long term storage in these containers. Do not allow the samples to freeze; store them in the garage storage room or the gear room if freezing nights are a possibility.
- Assemble the Dandy Dendy Concentrator (DDC) so the stopper can be removed without crushing any organisms and the drain will flow into the sink. Rinse the DDC thoroughly with tap water. Make sure the stopper is in place, and place a clean beaker or 60 ml sample jar under the insect spout to catch any leaks.
- Rinse the freezer container lid into the DDC with tap water. Transfer the dendy from the freezer container to a clean two-gallon bucket. The ethanol left in the freezer container can be poured into the DDC now or later. If there is a lot of particulate matter in the ethanol, wait till later to avoid clogging the mesh. Disassemble the dendy over the bucket, rinsing the vexar mesh squares, hardboards, and eyebolt into the bucket under running tap water. Place the mesh, hardboards, and eyebolt into a sample tray or dishpan. Leave the choreboy in the bucket to soak.
- Using a fine tipped forceps, pick all matter from the mesh pieces, hardboards, and eyebolt, placing it into the DDC. Pick everything even if you don't know what it is, or are sure it's not animal. Rinse all dendy parts again into the bucket, scraping the hardboard with a razor blade under running water. When everything but the choreboy has been picked clean and rinsed, remove the choreboy from the bucket and pour the rinse water through the DDC. Place the bucket below the tap and gently unfold the choreboy. Rinse thoroughly under running tap water, turning the choreboy inside out if the organisms don't rinse clean initially. Pick the choreboy clean with forceps. Pour the rinse water through the DDC and rinse the bucket, sample tray ,and freezer container into the DDC.
- Wash down the sides of the DDC with tap water. If there is a large amount of material in the DDC, use the forceps to gently transfer some of it to the sample jar. Use 70% ethanol to rinse the rest of the material into the sample jar. Rinse the DDC stopper and drain spout thoroughly. Label the sample jar in pencil with lake, dendy site number and letter, date set and date retrieved. Also record this information on a lab form and place it in the Dendy 3-ring binder. Fill the sample jar to the neck with 70% ethanol and cap tightly. Store all dendy samples from the same year together in a cardboard record storage box labeled with 'Dendy' and collection year. Transfer the sample box to the Zoology museum in Madison.
- Leave all dendy components out to dry, then store in plastic storage boxes. Hardboard pieces need to dry for a long time before storage to ensure they are dry throughout. Store the choreboys in a large plastic bag.

Note: To make 4L of 70% EtOH, mix 2950 ml 95%EtOH with 1050ml water.

1.0.4.1 Floating

The next step in the process would be to separate the plant material from the animal material. This is done by placing the picked material in a pan with a solution of sugar water, in which the animals will float and can be skimmed off the top for identification. Details of this procedure can be found in Anderson, R.O. A Modified Flotation Technique for Sorting Bottom Fauna Samples. Limnology and Oceanography. Vol. 4, pp. 223-225.

We do not float LTER samples at this time. They are stored as picked above.

1.0.5 Sampling Locations:

1.0.5.1 Trout Lake

- # 7 Three birch trees at water's edge with a fourth birch stump about 5 ft long hanging over the water, 15 ft. south of two maple (with a third broken-trunk of a maple). Set out approx. 75 feet. (Added in 1982)
- # 17 Approx. 200 ft. north of the old public access clearing by a double pine tree, half of which is broken off (w/ woodpecker holes). Also near a small dead birch tree about 20 ft. high.
- # 31 Set out from a 6 foot gap between 2 large clumps of cedar. Roughly the middle of the island shoreline, and just to the left (south) of the macrophyte site. (Added in 1982) (Description revised in 1993 to avoid the macrophyte site and make finding it easier.)
- # 67 45 ft. from shore, just south of the tip of the point, halfway to middle of small cove. Set out from largest white birch tree.
- # 50 Set out from west pine of the two large outstanding pines - note severe drop-off.
- **Pine broke off before retrieval in 1994.
- # 56 120 ft. from shoreline. Out from farthest to the west of 3 huge white pines. (not sampled in 1982-83) Deep Halfway between lab and Millers Island.

1.0.5.2 Allequash Lake

- # 14 Set 100 ft. from shoreline, out from group of white birch hanging over the water. Stump and large rock to the left.
- # 16 Set out from group of 3 white birch at shoreline; one birch uphill from two is broken and fallen between the two trunks.
- # 32 Set near overhanging black spruce to the right of all the big rocks and to the left of the dead bog trees.

Deep Permanent sampling site.

- # 40 Set to the left of the campsite, halfway between campsite and two overhanging birch trees, one dead and almost in water. (These birches are about 20 feet apart)

1.0.5.3 Sparkling Lake

- # 1 Off the boat landing to the south, set far enough out of the way so as not to be too obvious. Deep Permanent sampling site.
- # 19 Set between the two fallen trees.
- # 21 Set beneath large dead pine overhanging the water.
- # 24 By the last dead tree snag along the east shore, south of wayside. Set about 2 ft. to the south of the snag.

1.0.5.4 Big Muskellunge Lake

- # 82 Set 25 ft. from shore, out from a small birch tree near the water. 50 feet to the right is a large stump. Behind are several red pine.
- # 59 To the right of picnic area. Set at big oak 10 ft. from shore. An opening six feet wide (path?) is immediately next to the oak. Deep Permanent sampling site.
- # 51 By a stand of pines near a big double birch tree with one trunk dead, set out 10 feet from shore.
- # 43 Set out from dead log lying horizontal in the water, halfway between narrows and point to the south. There is a dead pine near the shore 40 feet to the right. (site redescribed in 1986 when no previous landmarks recognizable)

1.0.5.5 Crystal Lake

- # 6 Set out from 2nd pathway near quad of birch, east of boat landing.
- # 9 Set out from 5th pathway near a dead tree. One birch is immediately at left of path and four single birch are between paths 4 and 5.

Deep Permanent sampling site

- # 27 Near the north edge of the west beach area. Set out from large single birch, which is 30 ft. to right of trio of birch and an oak.
- # 43 Set out from two merging white pines (one trunk is smaller and behind larger one), near two small birch trees in a 'V'. (Campsite 27)

1.0.5.6 27-2 (Crystal) Bog

1.0.5.7 12-15 (Trout) Bog

- # 2 Mid Lake
- # 1 & - # 3 Approx. 200 feet to either side of walkway into the bog.

Part II

Biological Protocols

The following section contains protocols taken from https://lter.limnology.wisc.edu/research/protocols

2 Phytoplankton

Revised: Unknown

2.0.1 Pooling:

Label 250ml amber plastic bottles with preprinted labels. Add sample date to labels and cover with clear contact paper.

Fill out the pooling information form using the hypsometric tables to calculate % Lake Volume represented by each sample that has been collected. The last depth range always goes to bottom of lake. Check that '% lake volume' column totals 100. We want to create a 250 ml pooled sample, so the % lake volume numbers are multiplied by 2.5 to obtain the subsample volumes needed.

Invert sample bottle several times to mix. Use a graduated cylinder to measure out the required volume from each sample, and put it in the Pooled Sample jar. Rinse the graduated cylinder with RO water before pooling another lake.

Store pooled samples until the end of the year. Discard the remainder of the pre-pooled samples.

Send pooled samples to Phycotech the following spring, once danger of freezing during UPS transport is past. At Phycotech they are made into permanent slide mounts. We archive the slides in the UW Zoology museum. We do not archive any wet phytoplankton samples.

2.0.2 Slide mounting and counting

PhycoTech is the only commercial lab in North America to utilize a unique proprietary permanent mounting technique for archiving and preparing samples for enumeration. These mounts allow you to get further data at a later date, as well as maintain a permanent archive of the sample that is easily stored, maintains fluorescence, and does not degrade with time (100+ years). Permanent algal mounts allow archiving of diatoms AND soft algae. All periphyton samples to species include both HPMA mounts for the whole sample and Naphrax, acid cleaned mounts, for diatom identification to species level. Zooplankton samples are also permanently mounted using a slightly different process. Our algal taxonomist, Dr. Ann St. Amand, has over 20 years of experience and has processed over 13,000 periphyton, phytoplankton, bacteria

and zooplankton samples from both freshwater and marine systems. Dr. St. Amand is the only person who enumerates algal and zooplankton samples at PhycoTech, ensuring data integrity and consistency. Our In-house key and publication library numbers in the thousands, including the most current references. We have processed several state wide surveys in the Mid-West, West and Florida for phytoplankton and periphyton, each comprised of several hundreds of samples. PhycoTech also consults with Federal and State Agencies, including the Corps of Engineers, on experimental design and QA/QC issues. We process samples for general water quality, as well as the determination of exotic, toxic or taste and odor producing blue-green and chrysophyte algae. PhycoTech has extensive experience enumerating Prymesium parvum, a difficult to identify, toxic haptophyte from the Southwestern United States.

There are two state of the art microscopes used to process algal and zooplankton samples: an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Microfire digital camera attached, and an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film. For larger material PhycoTech also has a dissecting microscope. We have access to Notre Dame's SEM facility as well. Our computer network is a newly installed Novel Network connected to 6 new 2.4+ Gig workstations, with special adaptations for graphics and extra memory for efficiently handling our new data management system, ASA. All count related data is backed up daily to different media, with on-site and off-site copies weekly.

PhycoTech is now utilizing its proprietary data management software, ASA. This unique, powerful program not only tracks samples from receipt to data delivery within the same software program (every processing step is documented with initials and date, from Login to Analysis), but also provides significantly more information for each sample. With ASA, we are able to provide not only biovolume estimates, but volume and surface area estimates as well. Our biovolume, volume and area formulas are the most complete set available commercially, drawn from a variety of sources including current primary literature (See our Technical Approach), custom calculations designed in-house for complicated morphologies (e.g. Ceratium) and independently derived calculations from an Outside Engineering Firm that specializes in volumetric studies (e.g. area of a prolate, oblate ellipsoid). We also now provide data summaries on phyla, division, class down to taxa level automatically, depending on the analysis requested. In addition, our new program has the capability to calculate over 72 different diversity indices and summary statistics, including Shannon, Maragalef, Alpha and Berger_Parker Diversity measures, Species Richness and Evenness, Pollution Tolerance for diatoms, Environmental Tolerance for algae, Siltation Index for diatoms, Pollution Tolerance for diatoms, Palmer Index, ACC:CMN for diatoms, in addition to others. All taxonomic information from organism down to coloniality and structure is provided in the data set. All indices are calculated on an abundance (both Natural units/mL and Cells/mL) and total biovolume, biomass, volume and area basis, if biovolume/biomass is measured. QA/QC reports are generated

from within the program, comparing dominant taxa, reporting distribution checks and doing similarity calculations between the original sample and QA/QC sample. All slides are labeled with a unique Tracking ID code that appears on every report, data file and database generated within the laboratory.

Reports are provided in pdf format with summary graphics by group for each sample. Data files are provided in Excel format or other spreadsheet or database formats requested by the customer.

2.0.3 Phytoplankton Methods (HPMA)

The HPMA method for producing algal sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes (See references). Mounting distortion is minimal and the method provides the advantage of being able to go 100x to 1000x on the same specimen. Wet sample is always maintained in case clarification of identification is necessary. We strongly encourage our customers to use glutaraldehyde (final concentration of 0.25-0.50 %) for preservation of algal samples. It offers minimal distortion and allows the use of epifluorescence on algal samples while counting, which can dramatically improve the final results.

2.0.3.1 General protocol for making permanent algal mounts using HPMA equipment

Bunsen burner Beaker tongs Ice bath Pyrex beakers (150 ML) 2 Dropper bottles Mixed ester nitrocellulose filters (0.45 µm, 25 mm, plain) Glass slides (25 mm x 75 mm) Avery Laser Labels: #2181 Glass coverslips (25 mm x 25 mm, #1 or #1.5) Full view series support/drying racks (102 pin) Graduate cylinders Dumont forceps Glass filter towers (25 mL) Rubber stoppers (#2, #10) Filtration Manifold (6 station) Vacuum pump (plus appropriate plumbing, 25-50 mm Hg) Drying oven (60"C, not forced air) 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.

2.0.3.2 Method

SAMPLE:

1. Add enough glutaraldehyde to bring the final concentration to approximately 0.25% to 0.5% (for periphyton samples or "bloom tows", increase the final concentration to approximately 0.5%-1%). Keep the sample dark and refrigerate if possible.

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

RESIN:

1. Prepare ice bath in plastic tub.
2. Measure 25 mL of HPMA and 0.025 g of catalyst into a 150 mL beaker.
3. Deal with HPMA under hood and use gloves for both HPMA and catalyst.
4. 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 mixture is approximately the thickness of Kayro 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 usage. The entire procedure takes 2 to 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 to 48 hours twice; wash with soap and rinse with distilled water. Be careful to keep dust out of the beakers when making the resin. 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.)

2.0.3.3 Slides

MAKE THREE SLIDES FOR EACH SAMPLE – SHAKE SAMPLE WELL (100 TIMES-phyt. or 200 TIMES-peri.). Use Millipore 6-place stainless steel manifold and Millipore Filtration Towers. 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 filter towers. 3. Measure out phytoplankton sample using micropipetor or macropipetor (use graduate cylinder for very dilute samples, e.g. 30+ mL). For periphyton samples, remove sample with micropipetor (usually from 0.05-0.5 mL) and dilute to 10 mL in a graduated cylinder with distilled water. Agitate to mix. Choose sample volume so that each field at 200x contains approximately 20-30 cells. 4. Add sample to the tower and open valve. For periphyton samples or large phytoplankton samples using cylinders, rinse graduate cylinder into tower. Filter sample until water just clears the filter surface. Close valve and remove filtration tower just after the water disappears from the inner edge of the tower. 5. Place filter, FACE down, on a cover slip (# 1.5). Be careful to avoid bubbles under the filter. 6. Samples: 1. Samples preserved in glutaraldehyde: 3 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. 2. Samples preserved in lugols: 3 slides: Add 1-2 drops of the 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 to 24 hours.
8. Remove cover slips from oven. Add 1 drop 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 days. Make sure that the slides are completely polymerized before you store them or they will run and/or evaporate!!!! And believe me, its a mess!!!!
10. Label slides with ASA generated labels. All slides are labeled with the Tracking ID, which appears on all reports, data files and in all databases associated with that sample bottle and associated data.

2.0.3.4 Acid cleaning and making naphrax slides for diatoms

Phytoplankton/Periphyton:

If species identifications for diatoms are required or unknown diatom taxa are present, acid cleaned mounts in Naphrax are prepared according to the following procedure:

1. Take 5-20 mL of sample and transfer to a clean, 250 mL Pyrex beaker in the hood. Add room-temperature nitric acid to a total volume of 40-60 mL.
2. Cover with a watchglass.
3. After at least 24 hours has elapsed, carefully siphon off acid using glass siphon. Dilute acid and discard down drain with lots of extra water (Let water run for a minimum of 30 minutes after discarding acid).
4. A. Transfer remaining sample to a centrifuge tube and bring volume up to 14 mL with distilled water. Cap tube, mix well, and centrifuge at 3000 RPM for 5 minutes. Remove tube and carefully remove supernatant to the 2 mL volume marker with a micropipetor. Bring volume back up to 14 mL with distilled water, mix well, and repeat process. Complete a minimum of 6 centrifuge cycles. Check pH. If pH is lower than 7, repeat centrifuging process until the pH reaches 7.

- On the final cycle, remove supernatant to the 1 mL volume marker and bring volume back to 5 mL. Mix well to suspend pellet and decant into the storage bottle. Rinse the centrifuge tube 2 more times with 5 mL of distilled water and decant into the storage bottle. The total volume of the cleaned sample should be 15 mL. If the sample is very sparse, lower final volume.

5. Using a pasture pipette, transfer enough sample to a cover slip (#1, 22mm square) to cover the entire area and place in a vibration-free area until dry.

6. Add 1 small drop of Naphrax to the cover slip and invert onto a slide. Compress the coverslip with a clean object and place in an oven (60oC) for 1-3 hours, or finish on a hot plate.
7. Ring cover slip with fingernail polish and store.
8. Identify taxa at 1000x under oil immersion. Reference taxa are identified using a diamond scribing objective and permanent ink labels.

2.0.4 Quality Assurance Plan

2.0.4.1 Taxonomic Accuracy

Dr. Ann St. Amand, a senior level phycologist and taxonomic expert, will perform all phytoplankton, periphyton, and zooplankton identifications, enumerations, and biovolume/biomass measurements. Dr. St. Amand has published extensively in the area of algal ecology and has processed over thirteen-thousand algal and bacterial samples, and is qualified to analyze zooplankton and macroinvertebrates. Outside taxonomists will be utilized for taxonomic verifications when necessary.

All samples are initially test mounted for counting density before final mounting. Any major questionable IDs are noted in the database during counting, and indicated on the report as uncertain for taxonomic clarity. If enough sample is present, samples are sent out to other taxonomists for taxonomic confirmation. Distribution is checked on approximately every tenth sample, during the counting process. All biovolume calculations have been verified by comparing with current literature, and by comparing calculations using outside mathematical consultations.

2.0.4.2 Sample Custody

The chain-of-custody requirements for all laboratory operations for each sample (broadly interpreted to include procedures for the preparation of reagents or supplies which become an integral part of the sample, record keeping associated with sample acquisition, documentation of sample preservation, sample labeling, sample tracking to establish chain-of-custody, and shipping and packing) and laboratory analysis (i.e., laboratory coding, storage, check-out, and documentation of sample movement) will be fully documented in our data management software. Each sample received will be assigned an individual tracking number. The sample bottle, chain-of-custody, and sample log sheet which accompanies each sample sent are then used in conjunction with one another, to enter the samples individual tracking number and all available sample information, into our sample database, ASA. The database allows for quick and accurate tracking of each sample received by PhycoTech. Dated and initialed entries by appropriate personnel on all worksheets and in the log database are required for data validation. All information entered into ASA is fully QA/QC'd for content and accuracy. Sample receipt is confirmed with each customer. All slides are labeled with the Tracking ID, which

appears on all reports, data files and in all databases associated with that sample bottle and associated data.

2.0.5 Counting

2.0.5.1 Microscope:

There are two microscopes used to process algal samples: an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000x), Phase Optics (400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green, and UV excitation) and a trinocular head for photography, with a Microfire digital camera attached. For larger material PhycoTech also has a dissecting microscope. The BX60 is the primary microscope used for algal and zooplankton identification. There is also an Olympus BHT, research-grade compound microscope equipped with Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (400x), epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a Ricoh Camera Back attached using traditional slide and print film.

2.0.5.2 Data Entry:

Samples are enumerated within ASA directly. ASA is a database driven program with an integrated virtual TallyMeter module, containing over 130 databases. Up to 400 taxa can be enumerated within any one sample, and the entire database currently contains over 33,000 taxa, including algae, zooplankton, macroinvertebrates and bacteria. All calculations are completed within ASA, including concentrations, biovolumes, biomasses and diversity indices. Data files are also generated by ASA and saved in Excel format, while reports are formatted and saved to pdf format utilizing Microsoft Access, including summary graphics on a per sample basis. PhycoTech can then format data files in any format required by the customer, either horizontally or vertically oriented. QA/QC on counting is a recount done on approximately every 10th sample. ASA produces a QA/QC report comparing the original sample and the recount sample (quantitatively and qualitatively), including the distribution check. Samples pass that are within 10% of the QA/QC recount, quantitatively. Percent similarity may vary up to 20% on exceptionally diverse or sparse samples.

2.0.5.3 Phytoplankton:

The magnification used will depend on the size of the dominant taxa and the size and number of particulates. The goal is to count at multiple magnifications in order to correctly enumerate and identify taxa present that may vary by several orders of magnitude in size. If the sample is dominated by cells below 10-20 µm or the cells are fragile and difficult to identify, the majority of counting will be completed at 400x-1000x. Measuring for biovolume includes measuring

GALD and additional measurements including length, width and depth of different aspects of the colony or cell. ASA allows up to 28 separate measurements per taxa. Cell and colony shapes are approximated to a geometric figure and or figures and the appropriate calculations made. Currently, ASA has over 44 different shapes defined. From 10 up to a total of 30 natural units (sometimes higher on exceptionally variable taxa) are measured for each taxa depending on variability and number encountered.

2.0.5.4 Use ONE of the following methods depending on sample composition:

A. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae greater than 10-20 µm in GALD, count a minimum of 300 natural units and 15 fields at 200x (when possible, maximum of 100 fields). In addition, count taxa below 10 µm or fragile, difficult to identify taxa at 400x (minimum of 100 natural units and 10 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

B. DOMINATED BY SOFT ALGAE: If the sample is dominated by soft algae less than 10-20 µm in GALD or is dominated by fragile, difficult to identify taxa, count a minimum of 400 natural units and 15 fields at 400x (when possible, maximum of 100 fields). In addition, count taxa above 20-30 µm in GALD at 200x (minimum of 15 fields). Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

C. DOMINATED BY DIATOMS: If the sample is dominated by diatoms other than large, easily identified taxa (e.g. Asterionella), count a minimum of 15 fields at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, count soft algae according to size distribution (see A or B above) for a minimum of 15 fields at either 200x or 400x. Spread the number of fields counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting is completed when the standard error of the mean of the total number of natural units per field is less than 10%. For large taxa (200+ µm): scan at least one whole slide at 100x. This tiered counting method should yield a minimum of 400 natural units per sample (well over 400 cells per sample). Extremely sparse samples or samples with high particulates will yield less than 400 natural units.

NOTE: The goal, regardless of magnification, is to enumerate and identify a minimum of 400 natural units per sample exclusive of misc. microflagellates.

In the sample, random microscope fields are counted until AT LEAST 400 natural units (colonies, filaments, or single cells) of all organisms (total species) are encountered. Species counts are then made and average cell dimensions taken for biovolume calculations. For the colonies and filaments encountered, individual cells are counted, or estimated by subsampling in each natural unit encountered (e.g., by counting cells per unit of filament length and then multiplying by length of filament).

2.0.6 References

- Bergquist, A.M. 1985. Effects of herbivory on phytoplankton community composition, size structure and primary production. Ph. D. Dissertation. University of Notre Dame, Notre Dame, Indiana, USA.
- Crumpton, W.G. 1987. A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. Limnol. Oceanogr. 32: 1154-1159.
- St. Amand, A. 1990. Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. Dissertation. University of Notre Dame, Notre Dame, Indiana, USA.
- Olrik, K., et. al. 1998. Methods for Quantitative Assessment of Phytoplankton in Freshwaters, part I. Naturvårdsverket, Stockholm.

3 Zooplankton

Revised: Unknown

3.0.1 Purpose:

We collect zooplankton samples at the deepest part of the lake using two different gear types. We take one vertical tow with a Wisconsin Net (80um mesh), and a series of Schindler Patalas (53um mesh) samples spanning the water column. All samples are preserved in cold 95% EtOH.

After collection we combine subsamples of the individual Schindler Patalas trap samples to create one hypsometrically pooled sample for each lake/date. The individual depth samples are discarded after pooling except from one August sampling date per year. The Hypsometrically Pooled sample and the Wisconsin Net sample are archived in the UW Zoology museum.

We count zooplankton in one or two subsamples, each representing 1.8L of lake water, of the hypsometrically pooled samples to calculate zooplankton abundance. We count one sample date per month from the open water season, and the February ice cover sample. We identify individuals to genus or species, take length measurements, and count eggs and embryos.

Protocol log:

- 1981 – May1984 – a 0.5m high, 31L Schindler Patalas trap with 80um mesh net was used. Two Wisconsin Net tows were collected. Preservative was 12% buffered formalin.
- June1984 – changed to 53um mesh net on Schindler trap.
- July 1986 – began using the 2m high, 45L Schindler Patalas trap. Changed WI Net collection to take only one tow.
- 2001 – changed zooplankton preservative from 12% buffered formalin to 95% EtOH.
- The number of sample dates per year counted varies with lake and year, from 5 dates/year to 17 dates/year.
- 1981-1983 – pooled samples are of several types: Total Pooled (TP) were created using equal volume subsamples of the Schindler samples. Epi, Meta, Hypo pooled used equal volume subsamples from the Schindler samples collected from each of the thermal strata. Strata Pooled used equal volume subsamples from the Epi, Meta, Hypo pooled samples to create an entire lake sample. Hypsometrically Pooled (HP) is our standard, which uses subsample volumes weighted to represent the hypsometry of the lake.

3.0.2 Northern Highlands Lake District:

3.0.2.0.1 Jar Preparation

Label 4-ounce jars with computer-printed labels for the Hypsometrically Pooled and Wisconsin Net samples. Add the sample date to the label using a soft pencil. Cover the label with a strip of clear contact paper, making sure the contact paper completely covers the label and encircles the jar. Jar labels should contain the following information:

Lake Name Station # Lake Name Station # Day Month YYYY Day Month YYYY Hypsometrically pooled: surf.-bottom Wisconsin Net 80um mesh 2-m, 45 L. Schindler Patalas Trap Vertical Tow from Meters 53um mesh, Preserv: 95%EtOH Preservative: 95% EtOH

Weigh the empty jars, including lids, recording these weights on the 'Volume by Weight' data form. Fill the Schindler field collection and Wisconsin Net jars with 80 ml cold 95% EtOH, making sure that the Schindler collection jars have the correct lids. Leave the HP jar empty. Keep preservative-filled jars cold until the zooplankton samples are collected.

3.0.2.0.2 Sample Collection: Schindler-Patalas Trap

For LTER lakes use the 2-meter high, 45L Schindler-Patalas trap with 53um mesh net and cup. The volume of the trap used should be indicated on the Volume by Weight form.

Collect samples from the target depths at the deep sampling station in each lake. Sample depths are measured from the middle of the trap.

3.0.2.0.3 Target Depths:

- TR: 1, 3, 5, 7, 9, 15, 20, 27, 31 meters
- CR/BM: 1, 3, 5, 7, 9, 11, 13, 15, 18 meters
- SP: 1, 3, 5, 7, 9, 11, 13, 15, 17 meters
- AL/TB: 1, 3, 6 meters
- CB: 1 meter

Take samples starting at the surface and working down. Lower the trap slowly so that it remains vertical in the water. Pause at the target depth long enough to allow both trap doors to close completely, and check when it reaches the surface that both did close. Drain the trap through the net and cup, swirling the cup until the liquid level is below the mesh windows. Remove the cup from the net and pull out the center pin to drain the sample into jar, then rinse cup and pin several times with 95% EtOH into the sample jar.

3.0.2.1 Sample Collection: Wisconsin Net

Lower the net to the bottom sample depth. Pull it up slowly, at a rate of about 3 seconds per meter. A slow haul prevents the net from pushing water and plankton away from the mouth of the net. Drain the cup until the water level is below the lower window, then pour contents into the sample jar. Rinse the cup with 95% EtOH several times, adding the rinse to the sample jar.

3.0.2.2 Hypsometric Pooling

3.0.2.2.1 Rationale and Definition

In March 1986 the LTER Zooplankton Group decided to pool the discrete depth Schindler Patalas trap samples into one pooled sample per lake-date for counting. Counting pooled samples rather than all of the depth samples reduces the time to produce zooplankton count data. The group hoped to count pooled samples from the entire backlog of uncounted samples and eventually to count samples shortly after collection.

Samples are pooled considering lake hypsometry and, therefore, represent the entire lake. Previously, unpooled samples (2-9 samples per lake-date) or samples pooled considering only a water column were counted. Hypsometric pooling allows us to consider the zooplankton community as representing the entire lake, as our other limnological methods do, instead of just a column of water.

Lake hypsometry is a three dimensional image of a lake or basin. In a simplified example of hypsometry, a lake is similar to a cone filled with water. If the cone were divided into three layers by two equidistant horizontal planes, the volumes in those layers would be very different from each other. The uppermost layer would contain the most water. Similarly, the upper depths typically contain most of the volume of a lake.

Pooling is the creation of a new sample from subsamples of the Schindler trap samples collected from one lake-date. The volume of each subsample used to make the pooled sample reflects the depth range the sample represents and the volume of water that range represents relative to the entire lake volume. Samples pooled in this manner are called HP samples, for "hypsometrically pooled", and are referred to as "volume weighted" because the volume of lake water each depth sample represents determines the subsample size.

In sum, the advantages to this method of pooling are quicker turnover time and representation of the entire lake in a volume weighted fashion. Disadvantages of this method include the time required to pool subsamples, errors introduced during pooling, and the loss of more specific depth information.

3.0.2.2.2 Pooling Procedure

Allow the sample jars to air dry for a day or two. Weigh the Wisconsin Net sample and record the weight on the Volume by Weight form. Mark the liquid level on the jar with a 'Sharpee' brand permanent marker.

Add 95% EtOH to each Schindler trap sample to bring liquid volume up to a weight of 105g, measured by weighing the sample jar with lid on the balance. If sample jars already contain more than 105g of liquid, allow some of the volume to evaporate in the hood, and then bring up to 105g. Record the final weight of jar + sample + EtOH on the Volume by Weight form.

Calculate the subsample volumes, called 'target' volumes, using the hypsometric table for each lake. Record these volumes on the Volume by Weight form.

Mix the first sample gently and thoroughly by tilting the jar from side to side. Measure the target volume into a plastic graduated cylinder. Pour the subsample quickly and smoothly because the plankton settle out quite rapidly. Choose the smallest size graduated cylinder that can measure the target volume in one aliquot. Add the subsample to the labeled HP jar. Repeat with all other depth samples. When all of the subsamples have been added to the HP sample, rinse each graduated cylinder into the HP jar with several small volumes of EtOH.

Place the HP sample in the hood to evaporate the excess volume of EtOH. The final weight of the HP sample should be 105g. Mark the liquid level on the jar with a Sharpee brand permanent marker. If the samples are from the August quarterly, pour the remainder of each Schindler sample into a labeled jar for archival. For all other sample dates, discard what is left of the Schindler samples. Rinse and air dry the field sample jars.

Sample Storage and Record Keeping Store samples in cardboard records boxes obtained from UW Stores, storing samples from each lake in a separate box. Approximately one year of samples will fit in one box. Fill out the forms for each sample and sample box, as noted below.

Box Inventory Form: A record of box contents. It remains in the sample storage box. Volume by Weight Form: A record of samples collected for any one lake-date and their volumes, storage box number, and history of sample usage. Filed in 3-ring binders, one copy at the Zoology Museum and one copy with Corinna Gries. Samples Stored Form: A record of all samples collected and storage box number for each. Current forms are kept in a binder at Trout Lake; archived forms are kept in the UW Zoology Museum. The data are eventually entered into the electronic LTER Museum Catalog.

When boxes become full, check the contents against the Inventory Form and Samples Stored Form, and transfer them to the sample storage room in the garage. LTER samples and related paperwork are eventually transferred to the Zoology Museum at UW-Madison.

3.0.2.3 Zooplankton Counting

Before removing a subsample from any zooplankton sample jar, weigh the sample to check for evaporation. If the weight is within 0.1 gram of the last weight recorded on the Volume by Weight sheet, no fluid replacement is necessary. If the weight is more than 0.1 gram low, add 95% EtOH to the sample to bring it up to the correct volume.

Mix the sample well by turning the jar on its side and tipping back and forth gently. We use a Hensen-Stempel pipet with a 5-ml plunger for subsampling zooplankton samples. After mixing the sample, take the subsample as quickly as possible to avoid biasing the subsample as organisms begin to sink. There should be no air bubbles inside the Hensen-Stempel pipet. If there are, replace the subsample into the jar, completely dry the pipet, and begin again with the mixing. When you have a bubble-free subsample, dry the outside of the pipet and dispense the subsample into a cup with 53µ mesh bottom. Rinse the pipet into the cup with RO water, and continue rinsing the sample in the cup, washing the ethanol out of the sample through the mesh. Rinse the subsample into the counting tray with RO water, washing the mesh thoroughly to transfer all organisms into the tray.

After removing subsample(s) from the jar, weigh the sample jar, and record this weight in a new column of the Volume by Weight form. Record the balance used, your initials, and the date at the top of the column, and add a column header such as 'Column C minus subsamples removed for counting'. Do not put the subsample back into the sample jar after counting. Mark the new liquid level on the jar with a permanent marker. Replace the sample jar into the proper storage box.

Count copepods and cladocerans first, identifying individuals to species wherever possible, and staging all copepodids. Measure a subset of each species. Then add a few drops of Lugol's solution to the subsample to stain it, and count the rotifers and nauplii. Count two subsamples for copepods and cladocerans. Count one subsample for rotifers and nauplii. If there are less than 100 of the dominant rotifer in one subsample, count a second subsample for rotifers and nauplii. Add milli-RO water to the tray as necessary to keep the surface of the subsample level. If the surface becomes concave as the subsample evaporates, it is difficult to focus clearly, and measurements may become distorted.

Count all eggs attached to any species. For copepods and cladocerans, keep track of the number of individuals with eggs as well as the total number of eggs. Total number of eggs is sufficient for rotifers.

Measurements are done as follows: Measure copepods from the tip of the head to the end of the urosome, excluding the caudal rami. Measure cladocerans from the tip of the head to the posterior of the carapace, excluding tailspine. However, measure helmeted Daphnia species from the anterior edge of the eye to the posterior of the carapace. Rotifers are not routinely measured, but where they have been, the total body length excluding spines is measured. Body width rather than length is measured for Conochilus, Conochiloides, and Collotheca.

Describe, measure, and draw any unknown species on a separate sheet of paper. If possible, take a photograph of the unknown.

3.0.3 Yahara Lakes District:

3.0.3.1 Preparing the Sample

- Working under a hood, transfer the sample from the sample bottle into a 35 or 80 um mesh cup using a squirt bottle containing water. Rinse the zooplankton thoroughly with the water to get rid of any residue preservative (formalin or ethanol).
- Transfer the sample from the mesh cup into a beaker with marked volumes (measure and mark the beaker volume in 50 mL increments from 50-300 mL using a graduated cylinder). Using a squirt bottle containing water, make sure that all visible zooplankton are transferred from the mesh cup into the beaker.
- Fill the beaker with water to the 50 mL mark. If the sample contains a high density of zooplankton, particularly daphnia, it is desirable to dilute the sample further by adding more water to the beaker. A benchmark that has been used in the past is to dilute the sample so that there are at least a total of 50 of the dominant adult daphnia species in the 4 reps. Record the Total Volume of Sample on the data sheet.
- Using the 1 mL Hensen Stempel Pipette, stir the sample in the beaker in a figure-8 pattern. After becoming acquainted with how to use the pipette, take a 1 mL subsample and place it into a counting dish. A square counting dish with 36 square divisions has worked well for previously counted samples. Using a squirt bottle containing water, rinse the zooplankton from the pipette into the counting dish.
- Using a pointer, add a small drop of soap to the counting dish to break the surface tension and allow the sample to spread out in the dish. Add enough water so that the sample forms a continuous thin layer in the dish.

3.0.3.2 Counting the Sample

- Using the LTER Leica MZ8 dissecting scope, count the total number in the entire subsample of all individual cladoceran species as well as the largest cyclopoid species, Aglaodiaptomus clavipes. Use a benchtop counter to keep track of the number of individual species. It is easiest to keep the microscope at one power (perhaps 250x) and to scan the counting dish in both directions being careful to count the entire dish but not to overlap the passes so much that the same individual is counted twice.
- For the other species, a portion of the dish can be counted. Count individual squares in the dish (make sure that the dissecting scope is level and try to spread out the zooplankton evenly in the dish if they seem to be concentrated in one area). Record as a ratio the number of squares counted and the total squares in the dish (39.6) in the % Tray Counted column of the data sheet (see past data sheets). As a general rule, unless

there are very large numbers of a particular species of zooplankton, at least one third of the dish should be counted for each species of zooplankton.

- To measure individual species, set (and record on the data sheet in the Objective Magnification space) the scope to the 2.5 magnification setting. For each sample date (4 reps per sample date), measure 50 of each species of adult daphnia, 25 of each species of neonate daphnia as well as the unidentified neonate daphnia, and 15 of all other species (and eggs) found. Note that the shell spine is not measured on the daphnia and the setae are not measured on the copepods (see Balcer et. al). When measuring species, it is important to measure all individuals encountered until the specified number is reached so as not to bias the length measurements by inadvertently picking larger or smaller individuals. If the number of individuals counted of a particular species is less than the required number to measure, just measure those individuals counted. Record the lengths in scope units with 1.0 being 10 of the smallest units on the graticule (measuring device in the eyepiece).

- For those adult daphnia that are measured, note on the data sheet (in the manner specified on the data sheet): the number of males encountered, the daphnia which are exploded, and the number of eggs in the carapace for those daphnia which have eggs.
- Prepare another 1 mL subsample in the manner described above, and count the same percentage of the counting dish for each species of zooplankton as was done in the previous subsample(s) of the same sample. Count a total of 4, 1 mL subsamples per sample.
- Leptodora are counted from the entire sample. Dump the entire sample back into the mesh cup and rinse it (using a squirt bottle) into a counting dish. Count and record the total number of Leptodora in the sample. Using the 1x magnification (be sure to record 1x on the data sheet as the magnification at which Leptodora were measured), measure all Leptodora encountered (up to 50).

3.0.3.3 Storing the Sample

- After the sample has been counted, transfer it from the beaker and counting dish(s) back into the sample jar. Use a squirt bottle containing ethanol to carefully rinse all of the zooplankton back into the sample jar. Fill the sample jar with ethanol. On the sample jar, write 'Counted' , the initials of the person who counted the sample, and the date the sample was counted.

Entering the zooplankton data into an excel spreadsheet

- An excel spreadsheet including formulas which calculate density and mean length has been set up for the zooplankton data (zoopform1.xls and zoopform2.xls). Following are some explanations of the excel spreadsheet:
- Sample Volume (column F): This is the volume that the sample was diluted to in the beaker (Total Volume of Sample on the data sheet).

- Objective Magnification (measuring) (column G): This is the number that the zoom dial was turned to when measuring individuals. Note that this will normally be 2.5 except that for Leptodora it is usually 1.

- % Tray Counted (column N): This is the percentage of the dish that was counted for the particular species of zooplankton. Be sure that the entry is a percentage number and not the ratio written on the data sheet.
- # Per Tow (column O): This is a formula which calculates the total number of a particular species in the entire sample (or in the single tow). This is calculated using the following formula: ((Total Number in 4 Reps (M))/(4 mL))*(Sample Volume (F))*((100/(% tray counted (N). Note that because the entire sample is counted for Leptodora, the # Per Tow (O) formula is different for Leptodora (= column M). All other formulas in the spreadsheet are identical for all of the individual species.
- # Per m3 (column P): This is a formula which calculates the total number of a particular species in a cubic meter of water. This is calculated using the following formula: (# Per Tow (O))/((Tow Depth (E))p,(net hoop radius)2) where the net hoop radius for the Southern Lakes LTER net is 0.15 meters.
- # Species Measured (column R): This is a formula which counts the number of length entries made for an individual species.
- Average Length (mm) (column S): This is a formula which averages the individual species lengths in scope units and divides by the Objective Magnification (G). Note that this formula has been checked by calibrating the graticule in mm using a stage micrometer. If a different microscope is used or parts of the existing microscope are changed (objective lens (currently 1.0x planochromatic), graticule (currently 12mm:120) or eyepieces (currently 10x)), the graticule should be recalibrated and the formula adjusted accordingly.

3.0.3.4 Proofreading the zooplankton data

Following are some suggestions to avoid data entry errors - Check the zooplankton sample dates with the dates on the field sheets. If there is a discrepancy of a few days, assume that the date on the field sheet is the correct date. - After proofreading an entire lake and years worth of data (i.e. a complete spreadsheet), save the spreadsheet as a test file and sort by species. Check that all of the Objective Magnif. (column G) for Leptodora are 1 (if not check against the data sheet). Also make sure that the # Per Tow (column O) contains the same formula for all of the Leptodora entries (=column M). Also check the % Tray Counted (column N) and see if there are entries other than 100% for species which are normally counted in the entire tray (daphnia, aglaodiap, etc). It is also a good idea to sort the test file by other columns and look for irregularly high and low readings.

3.0.3.5 Southern Lakes LTER Zooplankton Species

Refer to the zooplankton data sheet for the list of species found in the southern LTER lakes. Note that there were some 1995 Lake Mendota zooplankton samples counted in 1996 for which a different protocol was followed.

- Certain zooplankton have not been identified to species because of the difficulty in distinguishing them from other similar species. This is the case with Leptodiaptomus siciloides, Leptodiaptomus minutus, and Skistodiaptomus oregonensis. Although it is possible to distinguish these species apart using a dissecting scope, due to time constraints it was decided to group them in the Diaptomus spp. category. Acanthocyclops spp., Chydorus, and Alona are other examples of categorizing to genus but not species.
- There was a change in counting protocol after the 1995 through 1999 Lake Mendota samples were counted. Initially, we used a size cut off of approximately 0.9mm (the size at which it becomes difficult with a dissecting scope to see the pectin of and thus speciate Daphnia pulicaria) below which we put all daphnia in a Daphnia spp. (neonate) category. For more recently counted samples, we have kept the same size cut-off at 0.9 mm to distinguish adult daphnia from neonates, but we have expanded the neonate category into Neonate Daphnia pulicaria, Neonate Daphnia mendotae, and Unid. Neonate Daphnia spp.

Refer to the LTER zooplankton notes for distinctive characteristics of zooplankton found in the LTER lakes. Useful references are listed below:

- Balcer, Mary D., Nancy L. Korda, and Stanley I. Dodson. 1984. Zooplankton of the Great Lakes, A Guide to the Identification and Ecology of the Common Crustacean Species. The University of Wisconsin Press.
- Dodson, Stanley I., Branchiopods and Calanoid Copepods of Wisconsin, 8/5/99 draft (or in press).
- Hudson, Patrick L., Janet W. Reid, Lynn T. Lesko, James H. Selgeby. 1998. Cyclopoid and Harpactacoid Copepods of the Laurentian Great Lakes. Ohio Biological Survey Bulletin NS 12(2):1-50.

Part III

Water Chemistry Protocols

The following sections contains water chemistry laboratory protocols.

4 Alkalinity

Revised: Grace Wilkinson, March 2023

4.0.1 Purpose:

This procedure describes the steps to potentiometrically titrate water samples with standardized hydrochloric acid to calculate alkalinity according to Andersen, 2002 and USGS National Field Manual for the Collection of Water-Quality Data. The units of alkalinity for this analysis are microequivalents of carbonate per liter.

4.0.2 Sample Holding Time:

14 days @ 4° C unpreserved

4.0.3 Materials Required for Titration

(see materials for regents in Section 4.5):

- MilliQ water
- 0.05N Hydrochloric Acid
- 1000 ueq/L Sodium Carbonate standard
- pH meter and probe
- pH buffers
- Analytical balance
- P200 manual, adjustable pipette
- P100 electronic, adjustable pipette
- Pipet tips
- Small, graduated cups for titration
- Micro-stirbar
- MilliQ squirt bottle
- Kimwipes

4.0.4 Glassware Preparation:

The glass jar to hold waste rinses and the graduated cups used for titration should be rinsed with DI water from the tap at the sink and set upside down to dry.

4.0.5 Personal Protective Equipment / Waste Disposal:

Nitrile gloves and eye protection should be worn while titrating. Always use chemical resistant gloves (not latex), safety glasses, lab coat, and a fume hood while using concentrated acids to prepare the 0.05N HCl. This is not only for your protection, but also to prevent contamination of samples. Proper personal protective equipment is always required for safety and contamination prevention.

4.0.6 Quality Assurance/Quality Control:

- Blind samples for analysis (i.e., field duplicates)
- Triplicate analysis of standard solution

4.0.7 Waste Disposal:

Most of the reagent solutions used in this procedure can go down the drain; however the pH should be near neutral (pH 5 – 8). Flush during and after disposal by running tap water. Excess dry reagents from preparing the stock can go in the trash.

Consumables Ordering: Item Catalog # Item Catalog # Na2CO3 salt Fisher AA3648522 Buffer 3.557 (Ricca) Fisher 149816 Optima HCl (500 mL) Fisher A466500 Buffer 6.87 (Ricca) Fisher 154016

4.0.8 Consumables Ordering:

Item	Catalog #	Item	Catalog #
Na_2CO_3 salt	Fisher AA3648522	Buffer 3.557 (Ricca)	Fisher 149816
Optima HCl (500 mL)	Fisher A466500	Buffer 6.87 (Ricca)	Fisher 154016

4.1 Preparing for Analysis

4.1.1 Remove samples for analysis from the fridge to allow them to warm up to room temperature prior to analysis.

4.1.2 Turn on the pH probe by pressing any key. Make sure it is reading in mV; if not, press 'MODE' until mV is being read. NOTE: put the meter on "Standby" after analysis

4.1.3 Prepare the pH probe for analysis. NOTE: follow these directions in reverse to store the pH probe after analysis

4.1.3.1 Remove the storage solution and parafilm from the probe

4.1.3.2 Check that there is enough liquid in the probe

4.1.3.3 Rinse the probe with MilliQ water from the squirt bottle and dab with a Kimwipe. NOTE: Do not rub the probe with a Kimwipe as this creates static.

4.1.4 Using the pH probe, measure the standard buffers (pH = 3.557 and 6.87) to create a calibration curve.

4.1.4.1 Place the pH probe in the buffer solution and allow the reading to stabilize. Record the millivolts (mV) on the sample data sheet.

4.1.4.2 Rinse off the pH probe with MilliQ water and dab dry with a Kimwipe

4.1.4.3 If the millivolts are not close to the values recorded below, the pH probe may be faulty or need recalibration. Consult with the lab manager before proceeding.

Buffer pH	millivolts
3.557	168
6.87	-19

4.1.5 Make sure that all the sample cups and stir bars are clean and dry.

4.1.6 Turn on the electronic micropipette.

4.2 Analysis of Standard

4.2.1 Place a sample cup with a microstir bar on the analytical balance. Tare the balance.

4.2.2 Pour and pipette 16 ± 0.1 mL of the 1000 µe/L standard into the sample cup on the balance. Remember, 16 mL = 16 g.

4.2.3 Record the mass of the standard sample on the standards data sheet.

4.2.4 Pour an aliquot of 0.5 N HCl into one of the plastic cups and cover with parafilm for storage. This will be the working acid solution you use for titrations and pre-dosing.

4.2.5 Pre-dose the standard sample with 0.2-0.4 mL of 0.5 N HCl acid. Remember, 100 µL = 0.1 mL

4.2.6 Place the standard sample on the stir plate and carefully position the pH probe so that is submerged in the sample without touching the sides or the microstir bar. Turn on the stir plate.

4.2.7 Using the electronic micropipette, add 0.01 mL aliquots of HCl until the meter reads 120 mV. Keep track of the volume added!

4.2.7.1 Record the pre-dosing volume and volume added in the following step along with the mV reading on line 1.

4.2.7.2 Do not record any readings or volumes until the mV reading is greater than 120 mV.

4.2.8 Add an additional 0.01 mL aliquot of acid to the standard sample and let the pH reading stabilize. Record the new volume and mV.

4.2.9 Repeat this process for 10 total aliquots of 0.01 mL of acid. Record the volume and mV reading each time.

4.2.10 Cleaning up the standard sample:

4.2.10.1 Dump the sample down the sink.

4.2.10.2 Rinse off the pH probe with MilliQ water and dab dry with a Kimwipe

4.2.11 Repeat steps from Section 4.2.1 - Section 4.2.10 twice more for a total of three standard sample readings.

4.2.12 Compare the amount of acid added to reach 120 mV and the mV readings thereafter. If the standard samples are wildly different from each

Table 4.3: Northern Lakes Pre-Dosing

Abbreviation	Lake Name	Pre-Dosing Volume
AL	Allequash Lake	0.2 mL
SP	Sparkling	0.2 mL
TR	Trout Lake	0.2 mL
BM	Big Muskellunge	0.1 mL
CR	Crystal lake	None
CB (Bog 27.2)	Crystal Bog	None
TB (Bog 12.15)	Trout Bog	None

Table 4.4: Southern Lakes Pre-Dosing

Abbreviation	Lake Name	Pre-Dosing Volume
FI	Fish Lake	0.7–0.9 mL
WI	Wingra	0.9–1.1 mL
ME	Mendota	0.9–1.1 mL
MO	Monona	0.9–1.1 mL
WA	Waubesa	0.9 mL
KE	Kegonsa	0.9 mL

4.4 Calculations

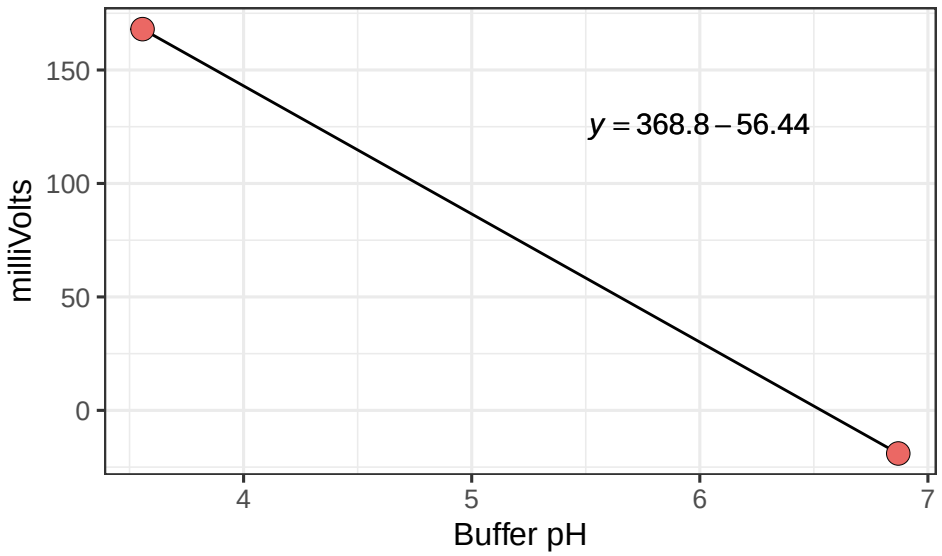
There is a calculation spreadsheet available on the shared drive. However, if the spreadsheet is not available or becomes corrupted, the steps below can be used to calculate alkalinity.

4.4.1 Use the calibration buffers to calculate a standard curve relating mV (millivolts) to pH. Calculate the slope (mstd) and intercept (bstd).

In this example, the slope (mstd) is -56.44 and the intercept (bstd) is 368.8. The slope should always be negative for the standard curve and the coefficients should be similar to the values in this example. If not, the pH probe may be faulty. Try calibrating.

4.4.2 Using the standard curve coefficients, calculate the pH for each point in the sample titration.

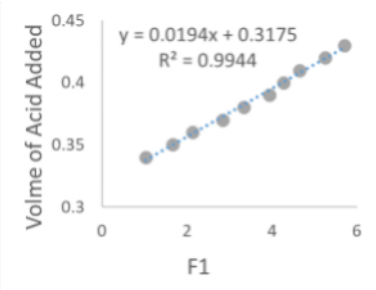
$$pH = \frac{(mV - \ b_{std})}{m_{std}}$$



4.4.3 The pH from the sample titration, the initial sample volume (mL), and volume of acid added (mL) are used to calculate F1 for each point in the sample titration.

$$F1 = 1000 \times \frac{VolumeAcidAdded + SampleVolume}{SampleVolume} \times 10^{-pH}$$

4.4.4 Regress the volume of acid added (mL) versus F1. The intercept (bF1) of this regression (mL) will be used to calculate alkalinity.



In this example, the intercept (bF1) is 0.3177. The slope should always be positive for this relationship and the R2 value should be >0.98. If not, the pH probe may be faulty, the pipette calibration is incorrect, or the analyst was not careful with pipetting. Try calibrating or reanalysis of the sample.

4.4.5 Calculate alkalinity (µEg/L) using molarity of the titration acid (nominally 0.05 N, but needs to be determined for each batch), initial sample volume (mL), and b_{F1}.

$$Alkalinity(\frac{Eq}{L}) = \frac{b_{F1} \times TitrantMolarity}{SampleVolume} \times 1e6$$

4.5 Preparation of Standards and Reagents

4.5.1 Preparation of 1000 µEq L^{-1} standard for alkalinity titration

Requires sodium carbonate (Na_2CO_3), 2 L of milliQ water, 2L volumetric flask, and standard solution storage bottles. The mathematical justification for the recipe below (note, there are 2 equivalents of Na+ per mol of Na_2CO_3):

$$0.1061g\ Na_2CO_3 \times \frac{1mol\ Na_2CO_3}{105.99g\ Na_2CO_3} \times \frac{1e6\ Eq}{Eq} \times \frac{2Eq}{mol} \times \frac{1}{2L} = \frac{1001\ Eq}{L}$$

4.5.1.1 Dry approximately 0.2 g of sodium carbonate (Na_2CO_3) salt in the drying oven for at least 3 hours prior making the standard

4.5.1.2 Label a 2 L volumetric flask and fill approximately half way with milliQ

4.5.1.3 Weigh out 0.1061 g of Na_2CO_3 salt using a weigh boat and the analytical balance. Add the salt to the volumetric flask with milliQ water (previous step)

4.5.1.4 Cap the volumetric flask with parafilm or the glass stopper, and swirl to dissolve and combine.

4.5.1.5 Fill the volumetric flask to the 2L mark with milliQ water and invert to mix.

4.5.1.6 Dispense the standard solution into the clean and labeled standard storage solution bottles. NOTE: the label should include the chemical composition, date of preparation, and preparer's initials.

4.5.2 Preparing 0.05 M HCl titration solution or alkalinity titration

Requires Fisher Brand Optima HCl, milliQ water, and a 1 L volumetric flask. Note, that for this solution, molarity is equivalent to normality because there is 1 H+ per Cl-. The mathematical justification for the recipe below:

For 100 g of solution, there will be 34 g of HCl, therefore:

$$34g\ HCl \times \frac{1mol\ HCl}{36.4611g\ HCl} = 0.9325mol\ HCl$$

The specific gravity (density) of HCl is 1.18 g/mL:

$$100g\ solution \times \frac{1mL\ solution}{1.18g\ HCl} \times \frac{1L\ solution}{1000mL} = 0.0847L\ solution$$

$$\frac{0.9325mol\ HCl}{0.0847L\ solution} = 11.01M\ (mols/L)$$

Based upon M1V1 = M2V2:

$$V1 = \frac{0.05\ HCl \times 1000mL}{11.01M} = 4.54mL$$

4.5.2.1 Fill the volumetric flask with ~800 mL of milliQ water.

4.5.2.2 Using a pipette, dispense 4.54 mL of HCl into the volumetric flask

4.5.2.3 Fill the volumetric flask to the 1 L mark with milliQ water. Cap and invert to mix.

4.5.3 Standardizing the HCl titration solution

Requires titrating the 1000 µEq L^{-1} standard multiple times and the intercept of F1 ~ volume of acid added (Section 4.4.4) is averaged to determine molarity.

4.5.3.1 Following the methods in Part 2 of this protocol, titrate at least 10 standard solution samples of 16 mL.

4.5.3.2 Enter the data into the calculation spreadsheet (based on calculations in Section 4.4 of this protocol) and record the intercept.

4.5.3.3 Average the intercepts from the 10 titrations of standard solution and use in the following equation to calculate molarity (equivalent in this case to normality):

$$\frac{1000}{1e6} \times \frac{16mL}{avg.intercept} = molarity\ of\ HCl\ solution$$

4.6 References

National Field Manual for the Collection of Water-Quality Data: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chaps. A1-A9, Chap. A6.6 "Alkalinity and Acid Neutralizing Capacity"

Andersen, C. B., 2002, Understanding Carbonate Equilibria by Measuring Alkalinity in Experimental and Natural Systems, Journal of Geoscience Education, v. 50, p. 389 – 403.