NTL Research Protocols

NTL-LTER

2023-06-01

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Welcome

This is the website for NTL-LTER research protocols.

Acknowledgements

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Part I

Water Chemistry Protocols

The following sections contains water chemistry laboratory protocols.

1 Alkalinity

Revised: Grace Wilkinson, March 2023

1.0.1 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.

1.0.2 Sample Holding Time:

14 days @ 4° C unpreserved

1.0.3 Materials Required for Titration

(see materials for regents in Section 1.5):

- 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

1.0.4 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.

1.0.5 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.

1.0.6 Quality Assurance/Quality Control:

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

1.0.7 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

1.0.8 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

1.1 Preparing for Analysis

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

1.1.2 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

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

1.1.3.1 Remove the storage solution and parafilm from the probe

1.1.3.2 Check that there is enough liquid in the probe

1.1.3.3 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.

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

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

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

1.1.4.3 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

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

1.1.6 Turn on the electronic micropipette.

1.2 Analysis of Standard

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

1.2.2 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.

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

1.2.4 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.

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

1.2.6 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.

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

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

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

1.2.8 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.

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

1.2.10 Cleaning up the standard sample:

1.2.10.1 Dump the sample down the sink.

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

1.2.11 Repeat steps from Section 1.2.1 - Section 1.2.10 twice more for a total of three standard sample readings.

1.2.12 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 1.3: 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 1.4: 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

1.4 Calculations

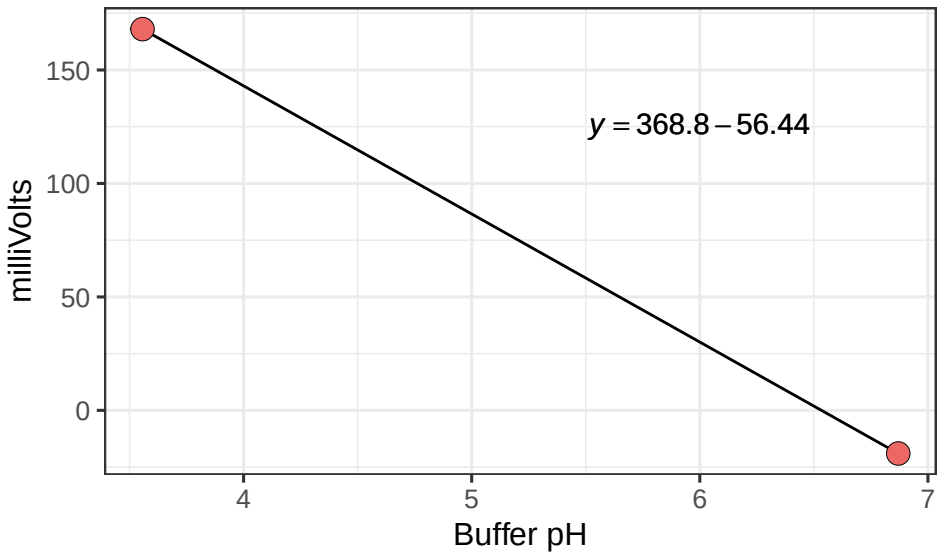
There is a calculation spreadsheet available on the shared drive. However, if the spreadsheet is not available or becomes corrupted, the steps below can be used to calculate alkalinity.

1.4.1 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.

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

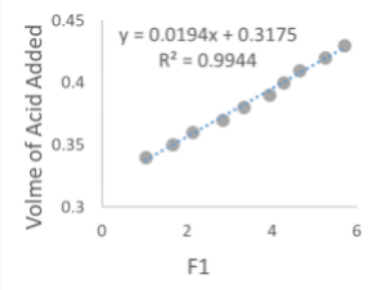
$$pH = \frac{(mV - \ b_{std})}{m_{std}}$$



1.4.3 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.

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

1.4.4 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.

1.4.5 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}.

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

1.5 Preparation of Standards and Reagents

1.5.1 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):

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

1.5.1.1 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

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

1.5.1.3 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)

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

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

1.5.1.6 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.

1.5.2 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:

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

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

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

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

1.5.3 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 1.4.4) is averaged to determine molarity.

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

1.5.3.2 Enter the data into the calculation spreadsheet (based on calculations in Section 1.4 of this protocol) and record the intercept.

1.5.3.3 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):

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

1.6 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.

Part II

Old Protocols

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

2 Ammonia and Nitrate/Nitrite

2.0.1 NITRATE/NITRITE

This automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

2.0.2 AMMONIA

This automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.

2.0.3 AUTOANALYZER REAGENTS

2.0.3.1 Nitrate/Nitrite

Ammonium Chloride-EDTA Buffer Reagent Ammonium Chloride (NH4Cl) 85 g MQ Water 1000 mL Disodium Ethylenediamine Tetraacetate (disodium EDTA) (C10H14N2Na2O8 $\cdot$ 2H2O) 0.5 mL Ammonium hydroxide(NH4OH) 6.5 mL (found in hood in acid washing room) TX10 0.05 mL
–Dissolve Ammonium chloride and EDTA in 900 mL Milli-Q water. Add Ammonium hydroxide and dilute to 1 L. Add TX10 to working reagent

3 Autoanalyzer Operating Instructions

Revision: 8/24/95 by James Thoyre

Start up:

NOTE: To save time, start a warm water bath to bring the reagents up to room temp. Takes about 15 minutes to get hot water and about 20 minutes to warm the reagents.

1) Hook up the appropriate analysis module to the colorimeter and the autosampler lines. Hook up bubble lines to air pumping lines on the peristaltic pump. Check the pump tubing diagrams in the Lab Methods Manual if you have any questions.
2) Place the appropriate pair of filters in the colorimeter. Only the colorimeter in the back will ever need to have the filters changed. The Nitrite/Nitrate module uses the 550 nm filter for both Nitrite/Nitrate and Total Nitrogen analyses. The Silica module uses the 660nm filters; the ammonia module uses the 630 nm filters; the phosphorus module uses the 880 nm filters.
3) Fill the Milli-Q reservoir 1/2 full with fresh Milli-Q water.
4) Check the waste carboy. Empty if it is 2/3 full.
5) Place the pump tubing reagent lines into MQ while the reagents are warming up. Place the sampler(probe) into the autosampler and let it run on MQ. Once the reagents have warmed up, move the lines from the MQ tothe appropriate reagent bottles (lines and bottles are labeled).
6) Turn on power switches at the outlet board. Be sure the autosampler's power button is not engaged.
7) Install the platen plate on the peristaltic pump assembly and run pump on high speed once to get reagents flowing through lines.
8) Check to see if all reagents are flowing smoothly through the pump lines. If not, change lines position on the pump rollers or replace worn pump tubing.
9) After the bubbles in the lines are flowing evenly and are uniform in size, turn on the colorimeter and plug in the heating element(when needed). Set STD. CAL. knob to value used on previous analyses.
10) After 15 minutes, turn on the strip chart recorder(s), and install the pen(s).
11) Use the baseline knob on the colorimeter to establish a zero baseline.
12) Once a stable baseline has been established(usually 45-60 minutes), place the prepared tray on the autosampler (a standard 1 and a Milli-Q sample should be run first for calibration purposes). Press down the autosampler power button.

13) When the calibration standard's peak comes through on the chart paper, adjust the STD. CAL. knob on the colorimeter to attain a peak height that is almost the full width of the chart paper at the peak's maxima. When the Milli-Q sample is being recorded, adjust the baseline knob of the colorimeter to place the recorder pen at its original zero position.
14) You now have the system calibrated and should not need to adjust the STD. CAL. knob the remainder of the analysis. If the baseline drifts up or down significantly over the course of the analysis, you may adjust it with the baseline knob when a Milli-Q sample is being detected. Record the adjustment with an arrow on the strip chart and label it with a message.
15) A full tray takes one hour and twenty minutes to be sampled completely. To assure that the autosampler stops at the end of the tray, place the red peg in the hole by the second-to-last sample cup. Continuous analysis may be maintained by taking the peg out and being sure to replace the sampled tray with an unsampled tray after the last sample has been aspirated.
16) Label the strip chart(s) with the name of the analysis, date, and analyst.

Shut down:

(Takes 30-40 minutes at end of analysis.) 0) Remove the cadmium column from the nitrate module without introducing any air into the column. 1) Remove reagent lines to a beaker filled with clean Milli-Q water. Run the pump on high speed one time. Place reagent bottles back in the walk-in cooler. 2) Remove the reagent lines to the small bottle half-filled with 0.1 N NaOH solution. Run the pump on high speed one time. 3) Remove the reagent lines to the beaker filled with Milli-Q water and run the pump one time on high speed. 4) Remove the reagent lines to a small bottle half-full of 0.1 N HCl. Run the pump on high speed one time. 5) Remove the reagent lines to the beaker of Milli-Q water and run the pump two or more times on high speed. 6) Remove the reagent lines to a plastic storage bag. Lift the autosampler "probe" out of the wash receptacle. Run the pump on high speed until liquid is no longer visible in the glass coils or the colorimeter tubing. 7) Remove the platen plate assembly and use a Kimwipe to clean the gray underside with isopropyl alcohol. 8) Turn off the power button on the autosampler and shut off all outlet power switches. 9) Turn off the power on the chart recorder and cap the recorder pen. 10) Check the waste carboy, empty if 2/3 full.

Additional Notes: If you are having problems with an unsteady or unusual baseline, the pump tubing and/or the pumping action are suspect. Most problems encountered are due to irregularities in the pumping action from worn pump tubing or blockages/air leaks in the lines. A level but noisy baseline may be caused by a small air bubble caught in the colorimeter's detection pathway. This may be "pulled through" by squeezing for a few seconds on the tube labelled TO PUMP and then releasing. A noisy baseline may also indicate worn pump tubing or a reagent which is precipitating. If you notice that liquid is dripping from the connecting lines of the mixing module and the colorimeter, you have probably forgotten to add a detergent (Aerosol 22 or Brij 35) to your reagents. See the Lab Methods Manual for details.

Tray Preparation for NO2&NO3/NH4 and N/ P Analyses: 1) Fill tray with conical-bottomed cups 2) Fill each cup completely with 1 N HCl. (83 mls conc. HCl/L) 3) Aspirate the acid from each cup. 4) Fill each cup completely with fresh Milli-Q water. 5) Aspirate the Milli-Q water from each cup. 6) Repeat steps 4 and 5. 7) Aspirate thoroughly any water droplets in the bottom or clinging to the sides of the cups. This step is very important! 8) Fill sample tray immediately with the samples.

Tray Preparation for BRSi and DRSi analysis: 1) Fill tray with flat-bottomed cups. 2) Fill the sample tray with samples. (No washes or rinses are needed!)

4 Benthic Macroinvertebrates

4.0.1 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.

4.0.2 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.

4.0.2.1 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.

4.0.2.2 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.

4.0.2.3 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

4.0.3 Equipment list for fieldwork

4.0.3.1 Setting:

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

4.0.3.2 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

4.0.4 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.

4.0.4.1 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.

4.0.5 Sampling Locations:

4.0.5.1 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.

4.0.5.2 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)

4.0.5.3 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.

4.0.5.4 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)

4.0.5.5 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)

4.0.5.6 27-2 (Crystal) Bog

4.0.5.7 12-15 (Trout) Bog

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