SAMPLING PROTOCOLS (1988-1993)

OF THE

POCONO COMPARATIVE LAKES PROGRAM

Robert E. Moeller Craig E. Williamson John A. Aufderheide Eugina M. Novak

POCONO COMPARATIVE LAKES PROGRAM Lehigh University

Department of Earth and Environmental Sciences 31 Williams Drive Bethlehem, Pennsylvania 18015

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OVERVIEW OF SAMPLING AND ANALYSIS

The sampling and analysis methods used for the routine monitoring of Lakes Giles, Lacawac, and Waynewood during 1988-93 are summarized here. This documentation is designed to allow evaluation and replication of procedures in the future. The methods are not necessarily the best available, and are not presented here as a manual of recommended techniques, although we have been generally satisfied with them. The descriptions assume familiarity with the equipment (i.e. with manufacturers' operating instructions for pH meter, oxygen-temperature meter, light meter, and fluorometer). We include notes on problems and on changes in procedure introduced during the course of the study.

According to the monitoring plan, each lake was sampled monthly, or twice-monthly during the summer. The lakes were usually visited in late morning, sometimes in early afternoon, or occasionally in late afternoon. A second visit was made after dark, usually between 10 pm and midnight, to resample the zooplankton. Samples were collected from a rowboat, which was moored to a buoy anchored near the deepest part of the basin (for locations see maps in 1992 Annual Reports to the lake owners). The same station was used at each lake throughout the study, including winter, when samples were taken through holes chopped or sawed through the ice. Because the buoy was sometimes dragged a little away from its designated site, changes in maximum depth are not to be interpreted as seasonal changes in water level.

Temperature, dissolved oxygen, and light (including Secchi depth) were measured first, in situ at 1-metre intervals, since thermal stratification dictated sampling depths for other parameters. The water column was divided into three layers, "EPI", "META", and "HYPO", corresponding in summer to epilimnion (low temperature gradient), metalimnion (steep temperature gradient), and hypolimnion (low temperature gradient). The metalimnion was delimited as the region where temperature changed more than 1°C per metre depth. In spring and fall, when thermal stratification was weak or absent, the water-column was divided into three equal layers (by depth). Under ice in winter the "EPI" layer was the top metre, and the remaining water column was divided equally to give the "META" and "HYPO" layers on each date.

Zooplankton samples were collected by vertical hauls of a closing net through each layer, while water samples (for pH, alkalinity, chlorophyll and algae) were collected at the mid-depth (to the nearest metre) of each layer. Duplicate collections of zooplankton and water samples were made in quick succession from each layer. Zooplankton were resampled at night to reveal diel changes in vertical distribution, and to get better estimates of abundance for taxa that swim to the bottom during the day (e.g. *Chaoborus*).

Water samples were collected with a transparent 4-L acrylic Van Dorn bottle (nominal sampling depth was to middle of the ca. 60-cm tall bottle). A 500-ml polypropylene bottle was filled for pH and alkalinity, and a 1-L polypropylene bottle for chlorophyll and algae. These were kept chilled in a cooler for transport to the field laboratory. Zooplankton were collected with tandem closing nets designed by C. E. Williamson (unpublished--see sketch **Appendix I**). A single frame supported a 48-\mu mesh net (Wisconsin-style, mouth diameter 15 cm) and a 202-\mu mesh net (diameter 30 cm). Zooplankton were preserved immediately in 10% sugar-formalin, then placed in a chilled cooler for transport to the field laboratory. A single composite of subsamples from the replicate 1 and replicate 2 chlorophyll-algae bottles from each layer was prepared for algae (ca. 100 ml sample preserved in capped glass bottles with 1% acid Lugol's solution).

Temperature was measured with a submersible thermister and oxygen was measured polarometrically with the Clark-style electrode of a Yellow Springs Instrument Co. meter. Light was measured as the ratio of photosynthetically active radiation (PAR, 300-700 nm, in quanta) between successive 1-metre depth intervals using Licor submersible—sensors and a Licor 2-channel datalogger. From these ratios a depth-profile of light attenuation was calculated. Ross pH electrodes with an Orion pH meter were used to measure pH. Alkalinity was determined as microequivalents/L by Gran titration. Chlorophyll-a (total and corrected for pheopigment) was determined fluorimetrically (Sequoia-Turner—fluorometer) following overnight extraction of frozen filters (Gelman AE glass-fibre filters) in a mixture of 90% acetone and methanol (5:1 by volume). Chlorophyll samples were filtered and pH and alkalinity were determined in the field laboratory, usually within 3-12 hr of collection.

After counting, zooplankton samples were returned to their capped plastic containers. These are in indefinite storage at Lehigh University. Algae samples have not been counted (though a set of seasonal composites from each lake for three years has been sent away for analysis). These are also retained at Lehigh. All zooplankton data, as well as the physical-chemical and chlorophyll measurements, have been entered into an electronic database (Borland "Reflex" v. 2 [1989]) running on IBM-type microcomputers. Inquiries to examine the database should be addressed to: Dr. Craig E. Williamson, Department of Earth & Environmental Sciences, 31 Williams Dr., Lehigh University, Bethlehem, PA 18015 (telephone 215-758-3675; FAX 215-758-3677).

The following sections of this report present detailed protocols for the procedures used:

- 1. Dissolved Oxygen
- 2. Temperature
- 3. Light and Secchi Depth
- 4. pH
- 5. Alkalinity
- 6. Algal Chlorophyll-a
- 7. Zooplankton

In addition, Appendix I includes several datasheets or graphs illustrating the analysis procedures. Appendix II is a detailed list of the zooplankton taxa recorded in Lakes Giles, Lacawac and Waynewood during the course of this study.

1. DISSOLVED OXYGEN

A. Overview

Oxygen is measured at 1-metre intervals in the water column on each sampling date. We use a Yellow Springs Instrument Company (YSI) oxygen meter (Model 58 or 57) with Clark-type polarographic oxygen electrodes. The electrodes (YSI catalog # 5739) are temperature and pressure compensating. They do not have a motorized stirrer, so are read while rapidly agitating the electrode cable (raising and lowering it within a 10-cm amplitude).

B. Protocol--DISSOLVED OXYGEN (Method 10)

- 1. Verify electrode condition. Replace membrane and solution with YSI replacement supplies if there are bubbles under the membrane or if the membrane has not been changed in a couple of months.
- 2. Check battery.
- 3. Turn on meter 15-30 min before starting sampling.
- 4. Set salinity calibration to zero if meter has a salinity-compensating knob.
- 5. Calibrate meter in air, with electrode inserted tightly in its saturated water-vapor housing, out of direct sunlight or wind. Set meter to 100% saturation when a constant reading is obtained (it may take 5 min or more for reading to stabilize).
- 6. Read oxygen concentration (to the nearest 0.05 mg/L) in surface water and other depths while agitating the cable. It may take several minutes to get stable readings, especially when moving into low-oxygen water.
- 7. Recheck 100% calibration. Read with probe back in its housing. Alternatively, verify surface water oxygen concentration. Note any major problems on the datasheet.

C. Comments--Dissolved Oxygen

- 1. The calibration to 100% saturation in air can be problematic. The meter often drifts, with calibration changing during the course of sampling. Consequently the ± 0.03 mg/L accuracy specified by the manufacturer is not realized. The realized accuracy is probably ± 0.5 mg/L in well oxygenated waters. The error is potentially larger in low-oxygen waters (see note 2).
- 2. The elevation of the PCLP lakes is not taken into account when calibrating the instrument to 100% saturation in air. In effect it is calibrated for sea level. Values should be multiplied by 0.95 to correct to the ca. 430 m elevation of the lakes ASL (Giles 428 m, Lacawac 439 m, Waynewood 421 m).
- 3. In anoxic waters (deep hypolimnial waters in late summer) the meter typically "bottoms out" at some positive oxygen level, usually 0.2-0.6 mg/L but sometimes more than 1 mg/L. Even long equilibration (> 15 min) with agitation does not overcome this error. Poor electrode condition or operator impatience may aggravate the problem, but are not the main cause. Low oxygen readings must sometimes be interpreted as "zero". This can be guessed at by the context (e.g. constant but positive values through deep portion of hypolimnia of Waynewood and Lacawac in late summer), but in other cases (early summer, intermediate depths) low oxygen concentrations (0-2 mg/L) are difficult to interpret. Values that probably represent zero oxygen are assigned a code of "4" in the "Data Flag" field for oxygen error codes.

2. TEMPERATURE

A. Overview

Temperature is measured at 1-metre intervals throughout the water column using the thermister of a Yellow Springs Instrument Company (YSI) oxygen meter.

B. Protocol--TEMPERATURE (Method 10)

- 1. Turn on oxygen meter 5-15 minutes before starting to sample.
- 2. Zero meter.
- 3. Record air temperature (shade electrode from direct sun).
- 4. Record temperature to a tenth of a degree Celsius after equilibration at each sampling depth.

C. Comments--Temperature

a. The meter is not routinely standardized. Although it is capable of giving results reproducible to $0.2\,^{\circ}\text{C}$ on any given day, the overall accuracy is much less. Moreover, we use several combinations of meters and temperature/oxygen probes. Two meters were calibrated in a water bath with two probes (4 combinations) on 16 December 1991 against a high-quality mercury thermometer (Fisher Scientific ASTM 90 C, #804-050). The accuracy was $\pm 1\,^{\circ}\text{C}$ over the range 0-30 $^{\circ}\text{C}$. The error was fairly consistent ($\pm 0.3\,^{\circ}\text{C}$) for a particular meter/probe combination (e.g. consistency of $\pm 0.3\,^{\circ}\text{C}$ within a single depth-profile). The $\pm 1\,^{\circ}\text{C}$ inaccuracy sometimes shows up in the database as date-to-date irregularities in the summer trend of slowly increasing hypolimnial temperature.

A. Overview

Light penetration is measured in two ways: as the Secchi disk transparency and as the downward attenuation of visible light. The Secchi disk is a black-and-white quartered disk 20-cm in diameter that is lowered until it disappears (Welch 1948 p. 159). The depth of disappearance is a measure of water clarity or transparency, and is less in waters high in suspended particles or dissolved organic matter. We measure Secchi depth by observing the descent of the disk through a transparent acrylic-bottomed viewing box (ca. 15 by 25 cm) to reduce surface reflections. Light attenuation throughout the water column is also measured metre-by-metre, using flat-plate, cosine-corrected quantum sensors (Li-Cor Instruments, Lincoln, Nebraska: LI-1925A underwater quantum sensors and LI-1000 data logger).

The quantum sensors are manufactured to give a quantum response to photosynthetically active radiation ("PAR"); that is, an equal response per photon (quantum) regardless of wavelength within the 400-700 nm spectral band. Since mid-1989 we have used a dual-sensor instrument, with the data logger automatically monitoring two sensors exactly 1 metre apart. The data logger provides a reading of irradiance at one depth (as $\mu \text{Einst/m}^2\text{-sec}$) and simultaneously measures the ratio of irradiance between that depth and the metre above it. By simultaneously monitoring two sensors, we obtain metre-by-metre estimates of the downwelling attenuation coefficients that are relatively uneffected by changing cloud conditions.

B. Protocol--SECCHI DISK TRANSPARENCY

- 1. Lower Secchi disk while observing it through the viewing box. Note the depth of disappearance (to the nearest 0.1 m). Then lower it another metre.
- 2. Raise disk slowly toward the surface. Note the level of reappearance.
- Record the average of these two depths.

C. Protocol--LIGHT ATTENUATION (Method 12)

- 1. Check batteries of the Li-Cor data logger.
- 2. Attach sensors to input connectors and remove protective caps from the sensing cells.
- 3. **Configure datalogger.** Verify that proper coefficients for both sensors are in memory, or re-enter them.
- 4. Read light intensity at 10 cm. Record irradiance of lower cell when it is 10 cm below lake surface, on sunny side of boat. Hold cell away from boat to reduce shading.
- 5. Continue with greater depths. At each depth "z", record the irradiance of the lower sensor (data logger channel 2) and the ratio of irradiances between upper and lower sensors (Rz = [z-1]/z; Math channel of datalogger).

- 6. Disassemble for storage. After sampling, let cable and case dry thoroughly. It is best to store the case open, to prevent high humidity from penetrating the meter and condensing on the electronics.
- 7. Calculate light penetration. The percentage of 0.1-m PAR at depth z is calculated as: 100/(R1*R2...*Rz), where R1, R2...Rz are ratios of readings for the depth intervals up to and including z.
- 8. **Update database.** Enter both the ratios and the calculated attenuation profile (% of 0.1-m PAR) into the Reflex database.

D. Variants--Light

1. Single-sensor quantum method (Method 10: June-July 1989)

A Li-Cor meter with a single underwater quantum probe was used. The attenuation ratios were constructed from readings at successive depths. Percentage of 0.1-m PAR was calculated directly from irradiance values. Several profiles were averaged when cloudiness caused changing light conditions (alternatively, on some dates readings were standardized to those of a separate deck cell).

2. Photometer method (Method 9: 1988).

A photometer (Protomatic, Dexter, Michigan) equipped with a silicon photocell and hemispherical diffusing collector was used to measure light at metre depth-intervals. This commercial version of a prototype described by Rich and Wetzel (1969) measures illuminance in foot-candles. Replicate profiles were obtained (sometimes) when clouds caused unstable readings. Ratios and percentages of 0.1-m light were calculated from these readings.

E. Comments--Light

- 1. Accuracy of the two-sensor method depends on maintaining precise relative positioning (1.00 metre apart) and orientation (horizontal) of the sensors, and on identical response of the two meters to the same light conditions (e.g. to a clear or uniformly overcast sky). This must be checked occasionally. Deviations from uniform response can be offset by resetting the calibration value for one or the other sensor. The whole unit should be returned to the factory for recalibration every year or two.
- 2. A major problem with all the light readings is shading from the boat (or, in winter, from people and equipment standing around the hole through the ice). Careful technique can minimize the effect on clear days (sensors lowered on sunny side of boat), but shading is often unavoidable. The effect is exaggerated attenuation at near-surface meter intervals, succeeded by one or two intervals of decreased attenuation. In our database, irregular or changing attenuation ratios within the top 3 metres of the water column should be treated with caution.

- 3. The photometer method of 1988 gave results not fully comparable to those from the Li-Cor quantum sensors, because of differences in collector shape (hemispherical 2π vs. flat cosine-response) and wavelength sensitivity (500-600 nm peak sensitivity of silicon photocell vs balanced quantum response).
- 4. Use of the viewing box improves reproducibity of Secchi disk measurements, especially on bright days with a choppy water surface. Our standard limnological disk is small (20-cm)--a bigger oceanographic-style disk would probably give significantly greater transparency values in Lake Giles.

F. References--Light

Rich, P.H. and R.G. Wetzel. 1969. A simple, sensitive underwater photometer. Limnol. Oceanogr. 14:611-613.

Welch, P.S. 1948. Limnological Methods. McGraw-Hill, New York, New York.

A. Overview

Values of pH are obtained on each sampling date from the EPI, META and HYPO samples with an Orion model SA-250 meter (manufactured ca. 1989) and Orion Ross combination electrode with epoxy body. The meter is calibrated at pH 7.00 and pH 4.00 using commercial high ionic strength buffer solutions. Samples and buffers are read at laboratory temperature. Samples are read with gentle magnetic stirring, with or without added salt solution to increase ionic strength.

B. Protocol--pH (Method 12)

1. Sample Collection

Fill 500-ml polypropylene bottles from Van Dorn collections (exclude all bubbles). Return to laboratory in cooler, Ideally, samples should be processed within a couple of hours, as soon as they have equilibrated to lab temperature (on counter or in water bath). Keep cool/dark if they must be held longer.

2. Calibrate meter (fill in pH calibration sheet)

- a. Soak electrode in distilled water or dilute buffer for several hours or overnight. Lower sleeve from hole in electrode to assure free flow of electrolyte.
- b. Attach temperature-compensating probe and turn on meter.
- c. Calibrate at pH 7. Rinse electrode with distilled water, blot tip of electrode casing (not sensing glass!). Gently swirl a 50-ml beaker of standard pH 7 buffer around electrode, then stop. When reading has equilibrated, adjust pH and enter the value.
- d. Calibrate at pH 4. Repeat above procedure with pH 4.00 buffer.
- e. Read pH 7 again. Rinse electrode, then read the pH 7 buffer again. Repeat the calibration if it is off by >0.03 units.
- f. Read distilled water. Rinse electrode thoroughly and blot. Then read a sample (50-100 ml) of distilled water, with swirling or gentle stirring. A reading of 5.2-5.6 should be obtained, though it may take a while. If meter does not come to this range, the electrode may be in poor condition. Note this fact.
- g. Add KCI to the distilled water. Reread the same beaker of distilled water, with stirring, after adding salt solution (Orion pHix/pHisa at ca. 0.5ml/50 ml of sample). If reading fails to equilibrate within the range 5.2-5.6, make notation on main data sheet that pH readings are suspect. If new reading is more than 0.1 unit different from reading without salt, electrode may be

suboptimal and this fact should be recorded on datasheets.

3. Read samples

- a. Rinse electrode. First rinse electrode with distilled water (squirt bottle), then place into ca. 50 ml beaker of sample. Swirl, and allow to stand a minute or longer to equilibrate. Discard sample.
- b. Read sample. Without rerinsing electrode, immerse it in a second 50 ml sample. Swirl sample, then stop and record pH value after it eqilibrates. This is the unstirred reading. Then resume swirling (or magnetic stirring) and record this pH also.
- c. Add salt and reread sample. Add 0.5 ml pHix/pHisa solution (1-ml disposable syringe) and reread, with stirring.

4. Reread buffers

Read distilled water, pH 4 buffer, and pH 7 buffer. Record values on the pH calibration sheet to demonstrate stability of meter.

C. Variants--pH

1. pH measurement without salt addition (Method 11)

Measurements and meter calibration are as in Method 12 except that the salt solution is not added to samples.

2. pH measurement without electrode verification (Method 10)

Measurements and meter calibration are as in Method 11 (no salt added to samples) but in addition the electrode is not tested in distilled water after standardization in buffers, and the buffers are not always read after the samples to establish stability of meter performance.

D. Comments--pH

- 1. Addition of KCl solution--always pHiX (1990) then pHisa (1991-93) sold by Orion Research--assured consistent pH measurements. At first the protocol was to add KCl to Giles samples only, and other samples if electrode performance seemed suboptimal. Later it was added to all samples, even though electrode performance seemed adequate. Usually adding KCl had little effect (change of <0.1 pH unit), so later results should be consistent with earlier data. Adding KCl tends to eliminate differences between stirred and unstirred samples, giving a value similar to that of a stirred sample without KCl.
- 2. For Method 12, the values entered in the database are the pH readings with pHiX/pHisa added, or averages of pHiX/pHisa values and pH readings stirred without pHiX/pHisa. Averages often were entered when the readings seemed to differ only by analytical noise. For

methods 10 and 11, the stirred pH values were preferred, though these, too, were sometimes averaged with unstirred values when the variability was small.

- 3. The PCLP lakes have quite dilute waters. After standardizing in high ionic strength buffers it is necessary to thoroughly rinse and soak the electrode in distilled water or sample. By reading distilled water after the standards (to pH ca. 5.5), we try to assure that the electrode is performing properly and has been thoroughly rinsed of buffer. Nevertheless, it is especially important to allow an extra long, several-minute soak in the first of any sequence of samples. On two dates in early 1993 we tested the reliability of the usual standardization procedure--using high ionic strength buffers--by reading special low ionic strength buffers (Orion Research, pH 6.97 ± 0.03 and 4.10 ± 0.03) along with the lake samples. Readings were within the claimed accuracy of the standards. These tests were performed with an electrode that had been in use for eight months.
- 4. The electrode has been replaced at 1-2 year intervals when it becomes sluggish (ie., slow to read pH 5.2-5.6 in distilled water). The electrode is used only for lake samples, and is little used except for the routine PCLP monitoring. It is stored closed and capped when not in use.
- 5. Overall, replicate sample collections yield consistent pH's (usually within ± 0.05 units, especially when KCl is added). Given the precision of the standardization, which usually "drifts" no more than 0.05 pH unit during the course of measurements, the individual pH values are believed to be "accurate" to within ± 0.15 pH unit. The manufacturer's claimed potential accuracy of the meter itself is ± 0.01 pH unit.
- 6. Since the samples are measured after they have equilibrated to laboratory temperature, and often 6-12 hr after collection, they may not represent in situ conditions. Since pH is measured during stirring, it may have decreased as carbon dioxide is taken up (Waynewood summer epilimnial samples?) or increased as it is given off (most other samples).

5. ALKALINITY

A. Overview

Alkalinity is measured by potentiometric titration of 100-ml samples with dilute acid (0.1 or 0.01 N hydrochloric acid). The endpoint is located graphically or by regression of Gran-transformed data (Mackereth et al. 1978). The titration is monitored with the same pH meter/electrode combination used for pH measurements. Five to eight data points within the pH range 4.4 to 3.7 are required for Gran plots.

B. Protocol--ALKALINITY by Gran titration (Method 11)

1. Sample collection

Samples are taken from the same 500-ml polypropylene bottles used for pH. The alkalinity titrations should follow the pH readings, which are performed when the bottles are first opened. It is best to measure all pH's before starting alkalinities, in order to avoid contamination with titrant acid.

2. Prepare pH meter

The pH meter (Orion Research model SA-250 with Ross combination electrode) is set up and standardized as for pH measurements.

3. Prepare titrant

Make a 1/10 dilution of 0.1 N HCl stock solution (e.g. Fisher standard solution) by pipetting 5.00 ml into a 50-ml volumetric flask, then bringing to volume with distilled water. The 0.1 N titrant will be standardized and used for Lake Waynewood and occasionally Lake Lacawac samples. The 0.01 N titrant is assumed to be an accurate dilution of the 0.1 N stock; it will be used for Lake Giles and Lake Lacawac samples.

4. Sample determination

- a. Measure out sample. Fill a 100-ml volumetric flask with sample. Decant into 120-ml plastic titration cup. Insert electrode and temperature-compensating probe.
- b. Fill microburet. Draw titrant (0.01 N for Giles and Lacawac; 0.1 N for Waynewood) into 2-ml syringe-type microburet. Adjust to 0.000 mark and wipe tip. Insert tip below surface of sample.
- c. Titrate sample. Start magnetic stirrer and titrate to pH 4.5, being careful not to overshoot. Then record titrant volume (to 0.01 ml) and resultant pH at this point and at 5-8 subsequent points between pH 4.4 and pH 3.7.

- 5. Standardize the titrant (fill out the alkalinity calibration sheet).
 - a. Weigh out standard. Redry sodium carbonate (Na_2CO_3 , FW = 106 mg/mmole) at 105°C. Cool under dessication, then weigh out several 106 \pm 1-mg aliquots. Store these tightly capped in 2-ml plastic vials.
 - b. Prepare 5 mM solution (10 mEq/L). Add contents of one vial (1.00 millimole), with rinsing, to 100 ml of water in 200-ml volumetric flask, then bring to volume.
 - c. Titrate the standard. Pipette 3.00 ml of the 10 mEq/L standard into ca. 100 ml distilled water in the titration cup. Titrate this 30 microequivalent sample with the nominally 0.1 N HCl as for the lakewater samples. Repeat the titration to obtain three replicates.
 - d. Calculate the second Gran function: F2 = $[antilog(5-pH)]/(V_0 + v)$ for all points in the pH range 4.4-3.5. Here V_0 is the volume of sample (100 ml) and v is volume of titrant (ml) to the measured pH.
 - e. Calculate titrant strength ("N"). Plot F2 vs v to find x axis intercept, v'. Calculate N from v' for each replicate titration: N = 0.001*30/v', where 30 is the microequivalents of standard added and v' is titrant volume (ml) to the equivalence point. N should be within a few percent of 0.1 mEq/ml.
 - 6. Calculate alkalinities from Gran plots.
 - a. Enter data from titrations of samples and standard carbonate into a spreadsheet that can calculate linear regressions with y intercepts <use Quattro-Pro program XA3YYDDD.WQ1>.
 - b. Calculate the second Gran function: F2 = $[antilog(5-pH)]/(V_o + v)$ for all points in the pH range 4.4-3.5. Here V_o is the volume of sample (100 ml) and v is volume of titrant (ml) to the measured pH.
 - c. Calculate alkalinity. Plot F2 vs v. The intercept v' gives the alkalinity (A) as microequivalents/litre: $A = v'*N*1000/(0.001*V_0)$, where N is titrant normality (Eq/L or mEq/ml), v' is ml of titrant to equivalence point, V_0 is volume of sample in ml. Calculate v' as the y intercept of v regressed on F2 in Quattro-Pro program XA3YYDDD.WQ1. Note: v' is better calculated as the x axis intercept of the regression line of F2 on v. But with r^2 's of > 0.99 (as all reliable titrations should have) the difference is negligible.

C. Variants--ALKALINITY.

1. Titration to equivalence point (Method 10: 1989 some dates)

A titration similar to that for the Gran plot was carried out, except that it was not necessarily continued into the pH range 4.4-3.7 required for the Gran plot. The equivalence point was determined graphically as the inflection point of pH plotted against v. The commercial titrant solutions were not standardized when this method was in use.

2. Titration to fixed pH endpoint (Method 9: 1989 some dates)

The titration was to a fixed endpoint of pH 5.2. This approximation was used for some Lake Lacawac and Lake Giles samples for which the pH vs ml of titrant plot did not show a distinct inflection point. The endpoint was selected after examining many Gran titrations of Lake Lacawac epilimnial samples.

D. Comments--ALKALINITY.

- 1. The Gran titration (Method 11) should give more precise values for alkalinity than the methods used in 1989. For Lake Waynewood samples, method 10 (titration to the inflection point) is comparable in accuracy and precision to the Gran plot since inflection points are easy to locate. In Lake Lacawac samples and Lake Giles hypolimnetic samples, Method 10 is applicable but imprecise because the inflection point is hard to recognize. Only the Gran plot establishes the slight negative alkalinity characteristic of Lake Giles.
- 2. There is some evidence that the Gran titration and titration to the inflection point give different results. For Lake Lacawac samples from March 1990, the Gran titrations consistently gave alkalinities ca. 10% greater than Method 10. Lake Waynewood samples from this date were only 3% greater by Gran titration. These differences may reflect some buffering constituent other than bicarbonate.

E. Attachments--pH and ALKALINITY (see Appendix I)

- 1. pH/Alkalinity calibration sheet
- 2. pH/Alkalinity datasheet with example data for Gran plots.
- 3. Gran plots of epilimnion samples from all three lakes on 1-3 July 1991 (Figure A.I.1). In these plots, the X axis incorporates the calculation of alkalinity from "v", the volume of titrant. The x axis plots $v*N*1000/(0.001*V_0)$. Therefore the X axis intercept, v', is alkalinity in units of μ Eq/L.

F. Reference--ALKALINITY

Mackereth, F.J.H., J. Heron and J.F. Talling. 1978. Water Analysis: Some Revised Methods for Limnologists. Sci. Publ. 36. Freshwater Biol. Assoc., Ambleside, England. p. 52.

ALGAL CHLOROPHYLL-a

A. Overview

Chlorophyll-a is measured as an index of algal biomass in the water column. Water samples (0.5-1 L) are filtered onto glass-fiber filters (Gelman AE). These are frozen and stored, ideally not more than 1 month. The still-frozen filters are extracted overnight, without grinding, in a 5:1 (vol:vol) mixture of 90% alkaline acetone (4 drops of conc. ammonium hydroxide/L) and methanol. The extraction is carried out in a dark refrigerator (2-4°C).

The extract, or a suitable dilution, is read in a fluorometer before and after acidification to 0.03 N HCl, and chlorophyll-a (corrected for pheopigment) and pheophytin-a are calculated. The fluorometer is calibrated periodically (every 2-3 months) with dilutions of more concentrated extracts of a higher plant leaf that are read, undiluted, in a spectrophotometer for determination of chlorophyll-a.

The fluorometer is a Sequoia-TurnerTM model 112 fluorometer (ca. 1989 manufacture) equipped with F4T5/B lamp, red-sensitive photomultiplier, Corning 5-60 excitation filter and 2-64 emission filter. This lamp/filter combination gives an acid ratio of 2.1 with purified chlorophyll-a (Sigma Chemical Co.), though we use a ratio of 2.0 for algal samples. The extracts of higher plant leaves, which contain chlorophyll-b and other pigments, but little or no pheopigment, give ratios of ca. 1.8.

B. Protocol--CHLOROPHYLL-a (Method 12: mid-June 1990--1992)

- 1. Sample Collection and Filtration.
 - a.Collect duplicate water samples (e.g. from "EPI", "META", and "HYPO" depths) with a transparent plastic Van Dorn or Kemmerer bottle. Fill 1-litre polypropylene bottles, and keep these in a darkened cooler during sampling. Filter samples within 8 hr. If they must be kept overnight (only a few dates in the 1989-1992 database), refrigerate.
 - b. Filter whole water sample. Measure out 500 ml of freshly shaken sample (composite of replicate bottles) into a graduated cylinder or other calibrated bottle (accuracy of $\pm 3\%$). Filter at low vacuum (0.1-0.2 atmosphere) onto 47mm glass fiber filters (Gelman AE). These are the "whole" samples.
 - c. Prepare and filter size-fractionated sample. Measure out 500 ml of composite sample, and rinse gently through a 20- μ m mesh nitex screen. Refilter this filtrate and, separately, the algae rinsed off the screen (vigorous jets of distilled water from a squirt bottle) onto glass-fiber filters as above. These are the "<20 μ m" and ">20 μ m" samples.
 - d. Freeze all filters in individual snap-cap petri dishes, labelled on top and bottom. Wrap in foil and store frozen. Samples should be analyzed within 1 month.

2. Extraction and Fluorometric Determination

- a. Extraction. Place frozen filter in calibrated polypropylene centrifuge tube and add ca. 11 ml of the 90% acetone/methanol (5:1 vol:vol) solvent. Cap and place in refrigerator overnight.
- b. Adjust volume of extract. Remove tubes from refrigerator and warm to room temperature (in darkness!). Working in subdued light, bring volume up to calibration (e.g. top of meniscus to "12" ml mark in Corning 15-ml polypropylene tubes--this gives a volume of 11.5 ±0.2 ml).
- c. Centrifugation. Shake tubes, then centrifuge (10 min at 3/4-full speed on a clinical centrifuge). Hold tubes in darkness.
- d. Zero fluorometer on the appropriate scale (usually the least sensitive "1" scale) with a cuvette of solvent. Rezero between each sample. Use 1-cm diameter cuvettes (supplied with instrument, or use any borosilicate test tubes that fit--but calibrate instrument with the same tubes!).
- e. Read sample. Read sample or an appropriate dilution (e.g. 1/5), so that reading is at least 25 and not more than 100 (top of scale is 109). Make dilutions with adjustable pipettors (e.g. 1 ml sample plus 4 ml solvent). Serial dilutions of 1/400 may be necessary for some Waynewood and Lacawac hypolimnial samples. Pipettors should be calibrated by weighing out aliquots of distilled water (1.000 g/ml).
- f. Reread after acidification. Acidify sample in tube with 3 N HCl (1 drop/5 ml sample). Mix, then reread after 1-2 min.

3. Calibration of the Fluorometer.

- a. Prepare standard extract. Macerate a healthy leaf from lettuce or other higher plant at hand. Place in capped tube with solvent to extract (1-several hr in darkness). Use 90% alkaline acetone. If the acetone/methanol mixed solvent is used, the extinction coefficient for chlorophyll-a needs to be adjusted downward slightly (see Pechar 1987).
- b. Centrifuge extract and decant supernatant into new tube.
- c. Prepare a dilution to be read on least sensitive scale of fluorometer. For example, 0.5 ml to 200 ml of acetone/methanol solvent--dilution factor df equals 0.0025--may be appropriate for a parent extract that reads ca. 0.5 at 665 nm in 1-cm cell of spectrophotometer.
- d. Read dilution in fluorometer. Read 3-4 replicate samples (aliquots of dilution) before and after acidification. Calculate mean values for the fluorescence before acidification ("Reading") and the acidification ratio "R" (before/after).
- e. Read undiluted extract in spectrophotometer. Read 3-4 undiluted aliquots of original extract in 1-cm cell of spectrophotometer (Perkin-Elmer lambda 3, with bandwidth ≤1 nm), with a capped cell of solvent in the reference beam. Read at 750, 665, 663, 645, and 630 nm; reread 1-2 min after acidification (1 drop 3 N HCI/5 ml of sample--mix thoroughly). Run same

cell with solvent only to obtain cell blank. (Note that cells containing solvent should be in place in both cell holders when instrument is first turned on--then a baseline correction is automatically recorded that will bring cells close to matching.)

- f. Verify wavelength calibration. Run one aliquot of original extract at many wavelengths in range 550-750 nm to locate chlorophyll-a absorbance peak. A peak at 663 ± 1 nm verifies instrument monochrometer setting.
- g. Calculate chlorophyll-a for standard. Compute mean blank-corrected absorbances for each wavelength. Enter these values in a program to calculate corrected chlorophyll-a by the trichromatic equation of Strickland and Parsons (1972). [Use Quattro-Pro spreadsheet "FLUORCAL.WQ1".]
- h. Establish calibration. Calculate the calibration factor "F" that gives chlorophyll-a concentration in the parent extract (CHLA as μ g/cm³) per unit of fluorescence on scale 1: F = Reading/(CHLA*df). Usually, F is ca. 3000.
- i. Calculate chlorophyll-a for samples. Enter calibration factor, acid ratio (= 2.0, see comments), fluorescence of diluted sample before and after acidification, dilution factor, volume filtered (litres), and volume of extract (usually 11.5 ml) into Reflex database to calculate chlorophyll-a corrected for pheopigment (CHLAC), pheophytin-a (CHLAP) and total chlorophyll-a (CHLASUM--includes pheopigment).

C. Variants--CHLOROPHYLL-a.

1. Spectronic-20 method (CHLOROPHYLL-a Method 10: 1988)

In 1988 a simple spectrophotometric method modified from Lind (1974) was used. Whole water samples of 1-litre volume were filtered onto Gelman AE filters and frozen. The frozen filters were extracted in the refrigerator overnight, usually without prior grinding, in 7 ml of 90% acetone made basic with a slurry of magnesium carbonate. The centrifuged extract was read in a broad-bandwidth (20 nm) spectrophotometer (Spectronic 20) at 663 nm. Chlorophyll-a was calculated using an extinction coefficient of 11.9 (vs 13.4 suggested by Lind (1974) for 1-cm cuvette). By this technique, no correction is possible for phaeophytin, which, along with any chlorophyll-b, contributes to the apparent chlorophyll-a.

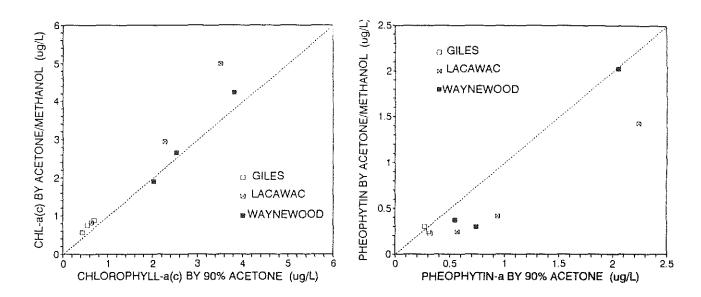
2. Extraction with grinding in 90% acetone (CHLOROPHYLL-a METHOD 11: 1989-mid June 1990)

Whole water samples of 1-litre were filtered onto Gelman AE filters and frozen. After 1-9 months these were ground in 90% basic acetone with a motorized teflon pestle in a glass mortar (Strickland and Parsons 1972, Wetzel and Likens 1991). The extracts were held overnight in the refrigerator, then read in the fluorometer. Chlorophyll-a and pheopigment were calculated as in Method 12.

D. Comments--CHLOROPHYLL-a

1. The Acetone/Methanol Extraction (Method 12). The acetone-methanol extraction modified from Pechar (1987) provides chlorophyll-a concentrations similar to, but slightly greater than, those of the traditional 90% acetone extraction with grinding (Method 11). We applied both methods to the 20 June 1990 EPI, META, and HYPO whole-water samples from the three lakes, after cutting frozen filters in two. As the graphs below show, the acetone/methanol combination extracted equal or greater amounts of chlorophyll-a (on average ca. 15% more). There was more pheophytin-a in some of the 90% acetone extractions, suggesting that part of the difference can be attributed to greater degradation of chlorophyll-a to pheopigment in the 90% acetone extraction. In any case, the extractions are close enough for direct comparability within our database. The acetone/methanol extraction seems to be both quicker (no grinding step) and analytically superior.

CHLOROPHYLL EXTRACTION TEST PCLP SAMPLES OF 20 JUNE 1990

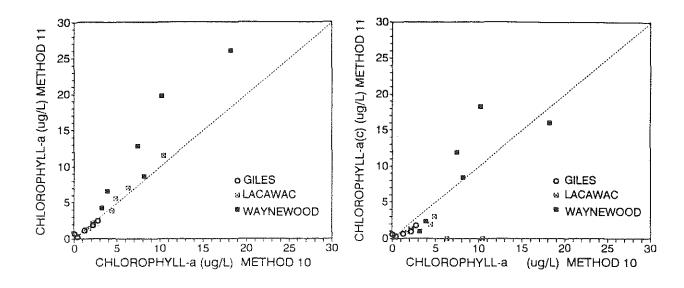


We verified that chlorophyll-a gives the same fluorescence yield and same acidification ratio in the two solvents (within ± 3 percent, the precision of our test). For greatest accuracy of calibration, the standard chlorophyll solution is prepared for spectrophotometric assay in 90% acetone, but the dilution for calibration of the fluorometer (usually 0.5/200 ml), is made up with the 90% acetone/methanol (5:1) mixed solvent.

Our overnight extraction in cold acetone/methanol (under refrigeration) substitutes for Pechar's extraction in boiling solvent. The techniques seem to be equivalent; no additional pigment was extracted by boiling our samples following the overnight extraction.

2. The 1988 Spectronic-20 Technique (Method 10). The 1988 chlorophyll values are not fully comparable analytically with the fluorometric, pheopigment-corrected measurements of subsequent years. This method was compared with Method 11, using samples from EPI, META, and HYPO of the three lakes from ca. 15 June and ca. 28 June 1989. Half of each filter was analyzed one way, half the other. The comparison between total chlorophyll-a (corrected chlorophyll-a plus pheophytin-a) by the fluorometric method and "chlorophyll-a" by the simple spectrophotometric method is plotted below:

CHLOROPHYLL ANALYSIS TEST PCLP SAMPLES OF JUNE 1989



Some high Waynewood samples (metalimnetic bluegreen populations) were underestimated by ca. 30%, but others were within ca. 10% of the fluorometric analyses. Epilimnetic samples from Giles were too low ($<0.5~\mu g/L$) to be accurately determined by the spectrophotometric method. Comparing the spectrophotometric values with values just of chlorophyll-a, corrected for pheopigment, gives a less satisfying picture: the high Waynewood samples remain underestimated by 30% (they lack pheopigment), but the other samples are consistently overestimated (by 20-100%). Values from 1988 should be compared to total chlorophyll-a data from subsequent years, not to corrected chlorophyll-a.

3. Fluorometer Calibration. Calibration factors obtained from extracts of plant leaf, which contain chlorophyll-b, are different from those obtained using purified chlorophyll-a (e.g. Sigma Chemical Co.). The factor is ca. 10% lower with pure chlorophyll-a, leading to higher apparent sample concentrations. The PCLP data almost always were calculated from leaf extracts. Analyses will be more accurate with samples that contain appreciable chlorophyll-b (e.g. lots of green algae). The acidification ratio for pure chlorophyll-a in the fluorometer is 2.1, but is 1.8-2.0 for leaf extracts free of pheopigment. We use a factor of 2.0 for all PCLP analyses.

- 4. Storage of Frozen Filters. Filters are stored in the freezer compartments of standard refrigerators. Long storage probably causes loss of pigment, or conversion of chlorophyll-a to pheopigment. Ideally analyses are carried out within 1 month. In 1989 and 1990, however, storage for 3-6 months, even 9 months, was the rule. Such long storage is noted in the error code field of the database.
- 5. Screening Samples with $20-\mu m$ Nitex. This procedure aims to separate large algae that may resist grazing from other, smaller types. A major problem is that the sum of the $<20-\mu m$ fraction and the $>20-\mu m$ fraction is usually less than the chlorophyll-a of the unfractionated duplicate. Sometimes the difference is quite large (20-50%). To avoid biasing the chlorophyll data, the sum of the fractionated sample is treated as a duplicate analysis only when it is $\ge 85\%$ of the whole-water sample. Size-fractionation was not done in 1988 or 1989. During summer 1990 it was done on alternate sampling dates, then every sampling date thereafter.
- 6. Interference in Lacawac hypolimnetic samples. During late summer and early fall, when the hypolimnion is anoxic, an interference prevents accurate measurement of chlorophyll-a and pheophytin-a by method 12. Upon acidification, fluorescence increases rapidly. All fluorescence is assigned to pheophytin-a; chlorophyll-a (corrected) is set to zero. But in fact some other pigment must be present, in high concentrations.
- E. Attachments--CHLOROPHYLL-a (see Appendix I)
 - 1. Chlorophyll Data Entry Sheet (example)
 - 2. Fluorometer Calibration Sheet (example)
- F. References--CHLOROPHYLL-a
 - Lind, O.T. 1974. Handbook of Common Methods in Limnology. C.V. Mosby Co., Saint Louis. 154 pp.
 - Pechar, L. 1987. Use of an acetone:methanol mixture for the extraction and spectrophotometric determination of chlorophyll-a in phytoplankton. Arch. Hydrobiol. Suppl. 78:99-117.
 - Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. 2nd. Ed. Fisheries Research Board of Canada, Ottawa. 310 pp.
 - Wetzel, R.G. and G.E. Likens. 1991. Limnological Analyses. Springer-Verlag, New York. 391 pp.

7. ZOOPLANKTON

A. Overview

Zooplankton are collected by vertical hauls of plankton nets at the main sampling station. Two nets with different mesh are used: (1) $48-\mu m$ (15-cm diameter mouth), which collects rotifers along with larger zooplankton, and (2) $202-\mu m$ (30-cm diameter mouth), which collects larger zooplankton only. The nets are mounted side-by-side and deployed simultaneously. The design of these custom-built nets is illustrated in Appendix I. These are "closing" nets (the $48-\mu m$ net is traditional Wisconsin-style) which can be supported alternatively from attachment rings at the net mouth (during deployment and sample collection through a portion of the water column), or from a lower ring below the solid Dacron collar (for withdrawal to the surface through a non-sampled layer). When the nets have been pulled to the top of the intended sampling layer, the attachment is switched from the upper to the lower collar by dropping a weighted messenger; this releases the upper ring, causing the collar to collapse and close off the net, which is then pulled to the surface. The tails of the nets, which are weighted down with lead sinkers, are sewn to small funnels equipped with tubing drains and screw clamps.

Zooplankton samples are collected both during the day, when other sampling is done, and at night (at least 2 hr after dark, usually between 10 pm and midnight). The water column is sampled in three intervals (epilimnion, metalimnion, hypolimnion during thermal stratification), so it is possible to examine day-night differences in vertical positioning of species within the water column. The nighttime samples tend to have higher concentrations of some species (especially *Chaoborus*), and are preferred for following long-term trends of species abundance (e.g., figures in the **Annual Reports** for water-column mean concentrations).

Zooplankton samples are killed and preserved by adding ca. 10 ml of chilled formalin (35-40% formaldehyde) containing sucrose (40 g/L) per 90 ml of concentrated sample. The 202- μ m net samples are briefly narcotized with carbon dioxide (rinsed in the net with soda water) before killing. Samples are stored at room temperature in tightly capped plastic cups before and following counting.

Appendix II is a list of taxa identified from the Pocono lakes, indicating also when each taxon was added to the suite of species consistently recognized and tallied by the counters. Not all taxa have been counted at the species level. Special effort has gone into species-level resolution of copepods and rotifers. *Daphnia*, in contrast, is counted only at the genus level. This list was prepared by John Aufderheide in April 1991, and was updated slightly by Robert Moeller in April 1993 with information provided by Gina Novak.

Calculations of concentration in the water column, or sampled portion of the water column, assume that the nets are 100% efficient at the nominal mouth area $(0.0177 \text{ or } 0.0707\text{-m}^2)$ for the organism in question (which may be counted from the $48\text{-}\mu\text{m}$ or the $202\text{-}\mu\text{m}$ sample, depending on its size). There have been several changes in counting strategy that may affect apparent trends. In general these changes (e.g. whether the $48\text{-}\mu\text{m}$ or $202\text{-}\mu\text{m}$ sample was used) were introduced to give more reliable and complete sampling.

Analysis strategy (since 1991):

a. From 202-μm mesh net collection: large Cladocera (all except *Bosmina*, *Chydorus*) and adult female cyclopoid copepods (except *Tropocyclops*). At least 100 organisms are counted in each of 1-2 subsamples.

b. From 48-μm mesh net collections: all remaining macrozooplankton (Bosmina, Chydorus, Chaoborus, Asplanchna, adult male cyclopoid copepods, adult male and female Tropocyclops, adult male and female calanoid copepods, cyclopoid copepodids, calanoid copepodids, copepod nauplii), rotifers, the ciliate cf. Rhabdostyla, miscellaneous other ciliates (an incomplete collection). At least 100 organisms (not including colonial cilates) are counted in each of 2 subsamples. Since 1991 copepodids have been counted at the species level.

During counting, an attempt is made to tally eggs, both loose and attached to adults.

B. Field Sampling Protocol--ZOOPLANKTON

- Prepare and rinse nets. Attach calibrated line, with messenger, to lower support frame and open clamps on discharge tubes. Lower and raise nets in surface water 2-3 times to rinse. Then close clamps and attach release mechanism to upper support frame.
- 2. Lower nets slowly enough that they sink backwards (mouth open and directed upwards) to the base of the sampling zone (1 metre above the bottom in the case of the deepest sample).
- 3. Collect sample. Quickly pull up nets to top of sampling zone, then drop messenger to close them (or pull through the lake surface for samples that extend to the surface).
- 4. Rinse sample into bottom funnel. Lower and raise mesh portion of nets 2-3 times to rinse organisms into bottom of net, then use squirt bottle of surface water to complete process.
- 5. Narcotize animals (202-µm sample). Pour soda water through mesh into sample within funnel, swirl and wait ca. 1 minute. Then drain away soda water through side of net.
- 6. Decant sample into plastic container. Rinse funnel contents into sample container (Sarstedt polypropylene urine specimen containers with screw caps), keeping volume below 100 ml.
- 7. Preserve samples. Add chilled sugar formalin (40 g sucrose per litre of formalin) to give a final concentration of ca. 10% formalin (ca. 2-3% formaldehyde). Cap containers tightly, verify labels, and place in cooler on ice for return to laboratory. Then store at room temperature in ventilated storage area.
- 8. Rerinse nets for next sample. If the next sample is a replicate haul from the same depth, the nets are not rinsed.
- 9. Clean nets following sampling. Back at the field station, thoroughly wash out nets with spray from hose, to reduce chance of cross introductions of species among lakes, and spurious records of contaminants in database. Allow nets to drip dry; then wrap up inside a plastic bag for storage.

C. Analysis Protocol--MICROZOOPLANKTON

- 1. Concentrate preserved sample. Partially immerse a small plastic cup with 20-µm mesh nitex across the bottom into the preserved sample, and withdraw organism-free solution until desired volume of organism-rich concentrate is reached (sometimes as little as 10 ml). Instead of a small cup, we use a cut-off turkey baster (a tube 2-cm in diameter) with 20-µm nitex mesh across the mouth. Carefully rinse organisms adhering to the underside of mesh back into sample. Record volume of concentrate (CV, in ml) using a graduated cylinder.
- 2. Prepare first subsample. Add 1.0 ml of well mixed concentrate to a Sedgewick-Rafter cell (5.0cm x 2.0cm x 0.1cm). Allow organisms to settle for 1-2 minutes before counting.
- 3. Count first subsample. Count one or more transects using a compound microscope at 100x. Organisms extending more than half way into the field are included. Count at least 100 organisms. Record "TV", the transect volume counted (TV is the product of number of transects, length of each transect, depth of cell, and width of field, all in cm). Rinse subsample into holding container.
- 4. Prepare and count second subsample. Then return both subsamples to original container. Add extra sugar formalin to compensate for rinse water diluted into sample. Cap tightly and return to storage.

D. Analysis Protocol--MACROZOOPLANKTON

The counting protocol is similar to that used for microzooplankton except for the following points:

- 1. The counting cell is a Bogorov chamber, with a total volume of 10.0 ml. It is counted under a dissecting microscope.
- 2. Samples are concentrated as for microzooplankton. The volume of concentrate (CV) is recorded. When organisms are relatively abundant the concentration is stopped with more than 20 ml remaining, so that two separate subsamples can be prepared and counted. When organisms are sparse, only a single 10-ml sample is prepared. The "transect volume" (TV) entered on the datasheet is the volume of sample added to the Bogorov chamber, which is always completely counted. If organisms are very abundant to begin with, or after concentration, a suitable volume "TV" (e.g. 2 or 5 ml) is pipeted into the chamber, with the difference (to 10 ml) added as tap water.

E. Attachments--ZOOPLANKTON (see Appendix I and II)

- 1. Microzooplankton Count Sheet (example)
- 2. Macrozooplankton Count Sheet (example)
- 3. Sketch of Closing Nets (Figure A.I.2)
- 4. Appendix II: List of Zooplankton Taxa Counted

APPENDIX I

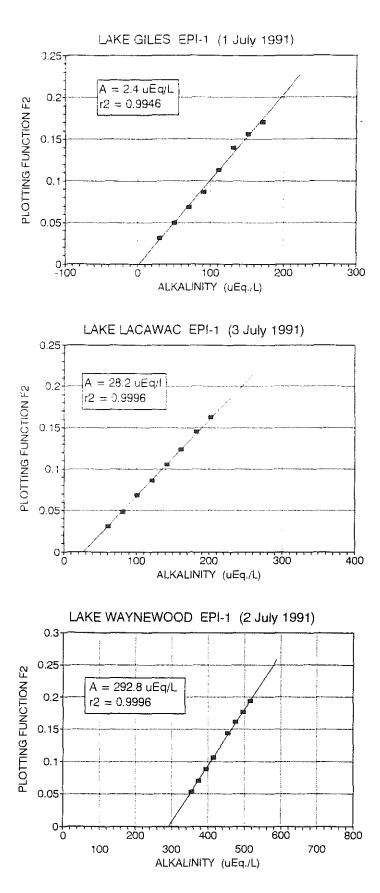


Figure A.I.1. Gran plots of alkalinity.
The x axis intercept gives the sample alkalinity in microequivalents per litre.

TLV

PCLP Project: ALKALINITY DATASHEET

composite Data: copied by R. Moeller 10/28/92 - Data for Gran plots.

Lake: Giles, Date: VII-1-91
Waynewood VII-2-91
Initials:

Sample Volume: pH-50 ml Acid Normality: 0.1(W); 0.01(G,L)

	Gi	les - EPI	Lacewa	c EPI	Waynewoo	od -EPI	
Layer:	. 1	EPI 2	1	FA2	1	2	
pH in bottle:							
pH Unstirred:	5.36	5,47	6.20	6.36	7.14	7.44	i age
pH Stirred:	5.32	5,40	6.35	6,33	7.39	7.50	Average these for values ase Jakabase
pH with pHIX:	5,34	5.41	6.33	6.34	7.36	7.47	Jalabas
Titrant N (mEglm) <0	01 N >	< <	0.01N>	<0.	in>	<u>.</u> .
Titrations:	0.50 4.30 0.70 4.16 0.90 4.06 1.10 3. 1.30 3.85 1.50 3.80	ml pH 0.30 4.51 0.50 4.30 0.70 4.18 0.90 4.07 1.10 3.99 1.30 3.92 1.50 3.86 1.70 3.80 1.90 3.74	ml pH 0.60 4.50 0.80 4.31 1.00 4.16 1.20 4.06 1.40 3.97 1.60 3.90 1.80 3.83 2.00 3.78	0.80 4.30 1.00 4.17 1.20 4.06 1.40 3.97 1.60 3.90 1.80 3.84	0.39 4.05 0.41 3.97 0.45 3.84 0.47 3.79	0.33 4.29 0.35 4.15 0.37 4.05 0.41 3.90 0.43 3.84 0.45 3.79	
Alkalinity (MEAIL)	(2.4)	-5.2	28.2	(27.6)	292.8	275.7)

NOTES: For Gran plots, 5-8 data points are needed in the range of pH 4.5 to 3.7.

Record <u>cumulative</u> volume of titrant added to each pH.

Check here if a pH/ALKALINITY CALIBRATION SHEET has been completed for this sampling.



PCLP Project: pH/ALKALINITY CALIBRATION SHEET

Date: VII-1-91 Initials: JAA, TLV

Recopied by REM 10/28/92

- I. pH CALIBRATION AND VERIFICATION (mix gently)
 - 1. Standardize meter at 2 pH's (e.g. 7,4)
 - 2. Reread buffers and record values; if necessary adjust and rerecord values:

3. Record pH in fresh distilled water (ca. 50 ml) after thoroughly rinsing the electrode:

5.61

(Read after equilibration; should be ca. 5.4-5.6)

Add 0.5 ml pHIX/50ml sample and reread: 5.50

4. Reread distilled water and buffers after samples:

$$DW = 5.65$$
 $pH 4 = 4.02$ $pH 7 = 7.00$

II. ALKALINITY CALIBRATION

This date 0.1 N =
$$0.1011$$

1. Dilute 0.1 N HCL stock to give a 0.01 N solution.

0.1 N aliquot volume (5) $\frac{5ml}{}$; final volume (50) $\frac{50ml}{}$.

2. Make up 0.01 N Na₂CO₃ (106 mg into 200 ml)

Vial # // ; final volume (200) 200 ml.

- 3. Titrate replicate diluted standards (add to ca. 100 ml DW).
 - 0.01 N Na₂CO₃ aliquot volume (3) 3.00 m.

A ml	pН
0.35	4.32
0.37	4.19
0.39	4.08
0.41	4.00
0.45	3.87
0.47	3.82
0.49	3.77
0.51	3.73

B ml	Нq
0.35	4.33
0.37	4.20
0.39	4.09
0.43	3.94
0.45	3.88
0.49	3.78
0.51	3,75
0.53	3.70

C ml	Hq
0.35	4.33
0.37	4.28
0.39	4.19
0.43	4.01
0.45	3.94
0.49	3.82
0.51	3.78
0.53	3.74

Calculated Normality:

O. 1018 N

(0.1021 N

M-26

0.0993 N

FLUOROMETRIC CHLOROPHYLL DETERMINATION

Anal	Lysis Da	ate: //2	22/9/	Calibration: Sample Da 3427 (R=2.00) 3 Septem							
Anal	lyst:	VSJ		34	27	(R	= 2.00)		3 Septen	iber 19	190
# .	Lake	Date	EMH	Time	Dep (m)	Filt Vol.	Filt 1½	Ext Vol	Dil	F1	F2 acid
/	GIL	9/3/90	EW			0.52	1	11.5	1/6	43.1	22.2
2			E420						3/8	27.2	15.6
3			E720						- 10st		
4			MW	100a 100					1/6*1/6	30.6	14.8
5			M 420						1/6	50.6	26.7
6			M>20						1/6 * 1/6	22.6	10.7
7			HW						1/6 * 3/8	37.2	19.8
8			H420						1/6	30.2	17.2
9	 	V	H>20			V	V	V	1/6	35,9	18.9
/0	LAC	9/3/90	EW			0.52	1	11.5	1/6	70.7	38.0
11			E420						1/6	37.0	19.9
12			E720		,				3/8	34.1	18.5
13			MW						1/6 * 3/8	49.5	30.1
14			M<20						1/6	77.6	52.9
15			M>20		·				1/6	52.9	27.1
16			HW						1/6 *1/6	45,0	57.4
17			H420						1/6*1/6	40.0	52.3
18	V	V	H>20			V	V	Y	1/1	37.6	27.3

Solvent: 90% Acetone/Methanol (5:1)
"Filt vol." is volume filtered in liters, "Filt vol." is volume junction ml.
"Ext vol" is extract volume in ml.
"Dil" is 1/df where df is dilution factor
M-27

FLUOROMETER CALIBRATION DATA SHEET

DATE: 28 May 1990	INITIALS: REM	
Chlorophyll Source Type:	X H (plant leaf) C (p	purified)

(Read 2/3 dilution of) Spectrophotometer Readings (at least 3 subsamples)
Extract

		Spec	Spectrophotometer Absorbances of Samples (without & with .4N HC)									
λ	BLANK	51	Sl a	S2	S2 a	53	S3 a	54	S4 a	Avg. w/o	Avg. w/a	
750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	NA	NA	0.000	0.000	
665	-0.005	0.742	0.442	0.740	0.427	0.745	0.447	NA	NA	0.742	0.444	
663	-0.005	0.773		0.771		0.775		NA		0.773		
645	-0.006	0.284		0.285	Ī —	MA		NA		0.285		
630	-0.010	0.139		0.142		NA		NA		0.141		

After reading the absorbances of 3-4 aliquots, run an additional aliquot through the spectrum range to pinpoint the chlorophyll peak:

λ (nm)	Absorbance	λ (nm)	Absorbance
750	0.000	665	0.745
690	0.015	664	0.765
685		663	0.775 -MAX
680	0.106	662	0.774
675		661	
670	0.517	660	0.740
668	0.624	655	0.555
666	0.712	650 652	0.445

Fluorometer sample readings (at least 3 samples)

Sample Dilution: 0.5ml/200ml = 1/400 (make sure the sample fluoresces above 35-40, adjust dilution accordingly)

	F1	F2 (acid)
S1	87.2	50.0
s2	87.8	50.2
s3	NA	NA
Average	87.5	50,1

Reduce to 2/3 for comparability: 59.4 33.4 CALCULATED CALIBRATION FACTOR (from fluorcal.wq1):

FROM FLUORCAL:

in 2/3 dilution, by

Strickland & Paesons

trichromatic equation:

Chlorophyll-2 0.826 µg/cm²

chlorophyll-b 0.211 n

(chlorophyll-c 0.010 ")

2827

(Extraction and Dilution is with 90% basic Acetone)

MICROZOOPLANKTON COUNTS

LAKE: Lacawac DEPTH (2):5-0m DATE: 1X-07-91

LAYER: Epi

TIME: DRY

STATION: A TI LAKE VOL. SAMPLED: 88.56 L LV=\pir^2z/10

COUNTING METHOD: Sedgwick-Rafter Cell

INVESTIGATOR: EMN DATE COUNTED: 12/3/91

NET MESH: 48µm

CONC. SAMPLE VOL.: 2041 TRANSECT VOLUME: 0. 104ml

DILUTION FACTOR: ___

_CV(ml) LV(l) * TV(ml)

INVESTIGATOR: EMN DATE COUNTED: 72/3/91												
		,,	JÄ	AR 1					JI	AR 2		
SUBSAMPLE		1			2			1			2	
TRANSECTS	1	2	3	1	2	3	1	2	3	1	2	3
Ascomorpha ovalis/species	4/5			$\frac{2}{3}$			5/ /5			4/7		
Asplanchna	0			0			0			0		
Collotheca mutabilis/species	%			%			2/0			1/0		
Conochilus solitary/colon.	00			0/2			1/2			0/		
Gastropus stylifer/hyptopus	1/0			1/6			1/0			0/0		
Kellicottia bostoniensis/ longispina	%			%			%			%		
Keratella cochlearis/crassa	5/0			6/			12/			10/0		
Keratella gracilis/hiemalis	%			%			0/			/0		
Keratella taurocephalus	2			5			2			2		
Lecane flexilis/mira	%			%			%			%		
Lecane luna/signifera	%			%			%			%		
Lophocharis species	0			0			0			0		
Monommata species	0			0			0			0		
Monostyla lunaris/ closterocerca	%			%			%			%		
Monostyla copeis	0			0			0			0		
Notholca squamula	0	<u></u>		0			0		İ	0		
Ploesoma truncatum	0			0	P***		0			0		
Polyarthra large/small	47/32			47/ 35			61/ /32			71/ /36		
Synchaeta	0			0			0	- Allbarra		0		
Testudinella parva/reflexa	%			%			1/0			%		
Trichocerca cylindrica/mult.	3/1			2/0			2/0			9/1		
Trichocerca similis/ rousseleti	%			3/0			4/0			%		

Trichocerca porcellus	0				0		0	
Rotifer eggs	29		16	· · · · · · · · · · · · · · · · · · ·	28		23	
Rhabdostyla	0		0		0		0	
Unknown Ciliates	76		119		130		153	
Tropocyclops prasinus ?/eggs	%		%		%		%	
Tropocyclops prasinus d	0		0		٥		٥	
Tropocyclops prasinus copep.	0		0		0		0	
Nauplii	/3		11		 20		<i>7</i> 7	
	<u>.</u>	 			 			
					 	 <u> </u>		
	<u> </u>							

LAKE: Lacawac DATE: X-07-9/ LAYER: Epi

TIME: DAY

MACROZOOPLANKTON COUNTS

LAKE: Lacawac DATE:/Y-O
DEPTH (z):5-O LAYER: Epi
STATION: A
LAKE VOL. SAMPLED: 353.43/88.36

DATE: 1x-07-91 LAYER: Epi

202 μm. // 48 μm CONC. SAMPLE VOL.: 50 / 50
TRANSECT VOLUME: 5 / 5
DILUTION FACTOR: CV(ml)
LV(1) * TV(ml)

LV=πr²z/10

COUNTING METHOD: Bogorov Chamber

INVESTIGATOR: TLV DATE COUNTED: #-25-72

			JAR 1			JAR 2				
	214	s	UBSAMPLE	es		SUBSAMPL	ES			
ORGANISMS	NET MESH	1	2	3	1	2	3			
Daphnia	202	99	100		106	116				
Daphnia (W)/eggs	202	21 34	21/35		22/34	10/19				
Diaphanosoma	202	0	0		0	0				
Diaphanosoma (W)/eggs	202	00	00		0/0	00				
Holopedium gibberum	202	16	15		21	15				
Holopeduim gibberum (W)/eggs	202	13/51	18/48		27 77	20 76				
Leptodora kindtii	202	0	/		0	0				
Leptodora kindtii (W)/eggs	202	00	%		00	00				
Chydorus	48	0	٥		٥	٥				
Chydorus (W)/eggs	48	00	00		00	0/0				
Bosmina	48	0	0		0	0				
Bosmina (W)/eggs	48	20	%		%	20				
Diaptomus minutus 9/eggs	48	30	5/22		46/145	10/32				
Diaptomus minutus đ	48	8	2	72	32	18				
Diaptomus minutus copepodids	48	19	18		75	32				
Mesocyclops edax 9/eggs	202	10	2/0		20	20				
Mesocyclops edax o	48	0	٥		0					
Mesocyclops edax copepodids	48	0	0			0				
Cyclops scutifer \$/eggs	202	00	%		00	00				
Cyclops scutifer d	48	0	0		۵	٥	Warner Williams			
Cyclops scutifer copepodids	48	0	0	AH-		0				
Ortho. modestus 9/eggs	202	20	%		40	60				
Orthocyclops modestus &	48				3					
Ortho. modestus copepodids	48	4	2		/3	4				
Chaoborus	48	0	0	Washing Co.						
Asplanchna	48	0	0		0		77000 db - 77200 db			

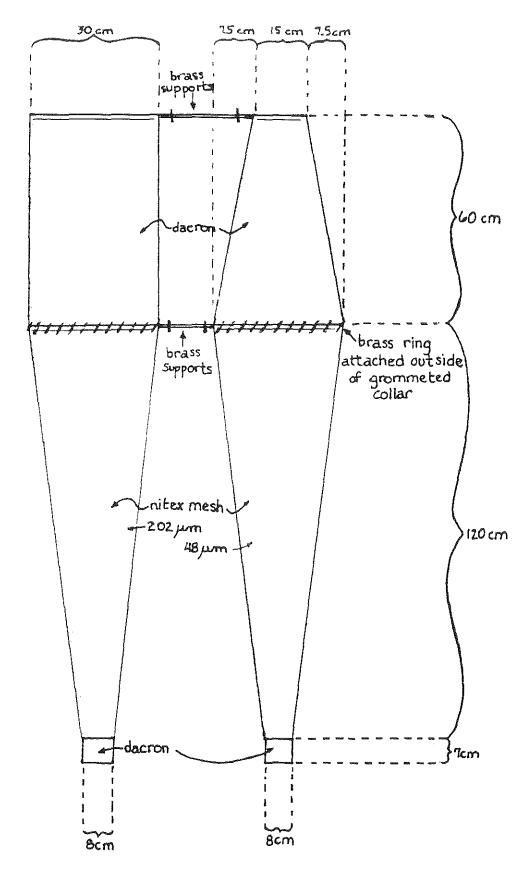


Figure A.I.2. Sketch of the closing nets used in 1989-1993.

The lower cones are of Nitex mesh, whereas the upper collars are solid dacron. Funnels with tubing for drains are sewn onto the small ends of the nets.

APPENDIX II: ZOOPLANKTON TAXA COUNTED

This listing of taxa was prepared by John Aufderheide in April 1991, and was edited slightly by Robert Moeller in April 1993. It documents several changes in the identification and counting of zooplankton that were introduced as the counters became more familiar with the material. Some simple changes have been transferred to the electronic database--at least to the annual summary datafiles--but others would require re-examination or recounting of earlier samples.

In 1991 we introduced additional changes in the counting strategy. Several macrozooplankton that had been counted from the $202-\mu m$ mesh samples were subsequently counted in the $48-\mu m$ samples. These changes were made to improve collection of smaller individuals, possibly at some lower collection efficiency for the larger individuals. In the following species list, therefore, several species are listed in the Macrozooplankton-- $202\mu m$ net category that more recently have been enumerated from the $48-\mu m$ net samples (see **ZOOPLANKTON** section).

Names and initials of investigators who identified species: John Aufderheide (JAA), Craig Williamson (CEW), Eugina Novak (EMN), Gabriella Grad (GG), Paul Stutzman (PLS), Natasha Vinogradova (NV).

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
		JAA	W	W9/3/90	RO:ANU
Ascomor pha ovalis This species is distinguishable from othe a thickened lorica consisting of a dorsal	Stemberger 1979 er species in the genus due to the presence of I and ventral plate.	JAA	G,L,W	W8/28/89	RO:ASC:OV
of dark circular bodies (typically four) v	Stemberger 1979 tinguishes this genus from others is the presence visible within the visceral mass. The general pha sp. from Gastropus sp.(see Gastropus sp.)	CEW	G,L,W	GLW6/7/88	RO:ASC
	Stemberger 1979 otifers (sandwich bags) which are typically 500 to ned from Synchaeta by the absence of a	CEW	L,W	1.W6/7/88	RO:ASP
The antennules of this species are large are nearly parallel to each other and cu than 500 um. Due to their small size, the made to distinguish between adults and	Edmondson 1959 en oval or rounded. The carapace valves cover the bode (compared to body size) and fixed to the carapace. The rive towards the body. The body is typically smaller ey are counted in the 48 um. samples. An attempt was i juveniles by counting the ones with eggs as Bosmina (small cladocera (Chydorus) was first started 8/28/89. 88 samples (CL:BOS:LO).	ney	L,W	LW7/19/88	CL:BOS
to calculate an egg ratio value we would	Edmondson 1959 nate the number of adults. We felt that in order d need to eliminate the juveniles from this calculation. eggs we would expect our egg ratio to be an overestima ther small cladocerans (see Chydorus).	JAA nte.	L,W		CL:BOS

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
(3.27) (3.27)		THIS ST LONGS			
come together to form a beak-like rostr	Edmondson 1959 counted in the 48 um samples. The fornices of the cara um which partially covers the anntenules. This t tends to be more ovoid. It is uncommon for us to find n-water samples.	•	G,L,W	G7/17/89 LW6/7/88	CL:CHY
Collotheca mutabilis This is the only species fully described f is the presence of long setae that emerg This species was initially mislabelled as		JAA	L,W	[.5/18/90 W7/18/90	RO:COL:MU
rotifer that secretes a gelatinous matrix. due to its accumulation of detritus. The	omic characterization while the organisms are	JAA	G,L,W	G10/9/89 L6/6/89 W7/26/88	RO:COL
at the ventromedian line, unlike most to ventral. There are three foot segments a foot segments. The shape of the torica i medial split. Due to these similarities th	Stemberger 1979 as of rotifer. The lorica of this species is split ricated species in which the lorica plates are dorsal or and two toes, unlike Lepadella species which display for s very similar to Lepadella triptera except for the is species may have been misidentified as a Lepadella si quently. Identification to species is difficult.		W	W10/11/91	RO:CLR
	Stemberger 1979 as. The colonies of Conochilus are surrounded ally smaller than 120 um in length (contracted). Chilus unicornis and C. hippocrepis.	JAA	G,L,W	G1.W6/7/88	RO:CON:CO
	Ruttner-Kolisko 1974 solitary species that secrete a gelatinous matrix. I lus based on discussions with CEW. Stemberger puts	JAA	G,L,W	G6/7/88 LW/8/28/89	RO:CON:SO

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
	Edmondson 1959 with the 48 um mesh since it is believed that The data show evidence that the 48 um mesh is nisms.	CEW	G,1.,W	GLW7/3/89	CY:CYC
	Edmondson 1959 they are now counted with the 48 um sample. net collections from 1989 suggested that many agh the 202 um mesh.	CEW	G,1.	GL7/3/89	CA:DIA:MI
Dia ptomus sp. copepodids These are copepodids of Diaptomus ore there is the possibility of them passing the	Edmondson 1959 gonesis. They are counted in the 48 um, mesh since grough the 202 um mesh	CEW	W	W7/3/89	CA:DIA
	Stemberger 1979 in the genus by the presence of a rectangular pecies is not as large as E. pellucida which is also in G id is ovoid in shape (150 um).	JAA iiles.	G	G10/19/90	RO.EUC:Pa
The lorica is almost spherical in shape wanteriorly. This is a large bodied rotifer (Stemberger 1979 notly keeled. The toes are long (>100 um) and slende ith a slight enlargement of the posterior end and poin (>290 um) and is the largest found in the PCLP lakes a a littoral tow in Lacawac, but none have been ten E. pellucida based on its large size.	ited	G	G7/19/90	RO:EUC:PI
-	Stemberger 1979 as used to identify this species. It was > 1.8. aynewood and is most likely to have made up as Filinia sp.	JAA	W	W7/18/90	RO:FIL:LO
Filinia sp. This genus was described only in Wayne caudal bristle were the key taxonomic fe	Stemberger 1979 wood. The two lateral bristles and the single atures used for identification.	JAA	W	W7/26/887/18/90	RO:FIL

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
Filinia terminalis Only one specimen of this species was for ratio of the lateral bristles: caudal bristles.	Stemberger 1979 ound. It was distiguished from F. longiseta by the es of <1.6. The LBL:CBL was 1.19.	JAA	W	W3/25/90	RO:FIL:TE
but has a prominent eye spot like a Sync as with G. stylifer. I went with G. hyptop	Stemberger 1979 ecies. It has the overall body shape of a Gastropus chaeta (see Synchaeta). The body is not as compressed pus from Stemberger's key. This species was a) and may have been placed with Synchaeta.	JAA	G,L,W	G9/24/89 LW11/12/89	RO:GAS:HY
Gastro pus sp. This is a genus of soft-bodied rotifers. Thowever, G.hyptopus is not very compr	Stemberger 1979 They can be laterally compressed (G.stylifer). essed and is similar to a Synchaeta.	CEW	G,L,W	GLW6/7/886/19/89	RO:GAS
Gastro pus stylifer This species is laterally compressed. In it shaped. This species tends to be colored	Stemberger 1979 ts contracted state it appears to be lemon , usually a shade of pink.	JAA	G,L,W	GLW6/19/89	RO:GAS:ST
Hexarthra mira So far recorded only on 5/13/92.	Stemberger 1979	EMN,NV	L ·	L5/13/92	RO:HEX:MI
	Stemberger 1979 gispina based on the presence of only four spine is typically longer than the other three.	CEW	1.,W	L7/12/88 W6/7/88	RO:KEL:BO
	Stemberger 1979 of unequal length. Typically three are longer cally found in the cold hypolimnetic waters.	CEW	G,L,W	GW6/7/88 I.6/28/88	RO:KEL:LO
	Stemberger 1979 single posterior spine exceeding body length and ngths (see K.bostoniensis and K.longispina).	CEW	G,1.	GL.6/7/886/28/88	RO:KEL

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
Keratella cochlearis This species typifies the Keratella genus. It has posterior spine. There are 1 or 2 pairs of polygo. The anteromedian ridges (beneath the anterom The lorica is typically translucent and not heavi Keratella sp.	onal facets on the mediodorsal surface. sedial spines) come to a point posteriorly.	JAA	L,W	I.W6/20/89	RO:KER:CO
V . 11					
Keratella crassa This is a large (150-200 um. body length) robust There are two pairs of distinct polygonal facets spine. It was originally counted as Keratella sp.	. This species only has a single posterior	JAA	1.,W	LW6/20/89	RO:KER:CR
Keratella earlinae This species is very similar to K.cochlearis in bo dorsal surface is developed posterior to two irre anteromedian surface are straight-lined, giving The lorica of this species is heavily pustulated a This species has been separated from K.cochlea	gular medial facets. The ridges of the the appearance of an open rectangle, and appears much darker than K.cochlearis.	JAA	W	W6/19/89	RO:KER:EA
Keratella gracilenta This species is similar to K.cochlearis in its gene of hexagonal facets on the mediodorsal surface pustulated. I went with Ahlstrom's classification americana (classified by Carlin 1943). Included It was present but not very abundant in 1989, an	t. The lorica of this species is heavily n. This species is also known as Keratella in K. cochlearis until August 13, 1990.	JAA	l.	L8/13/90	RO:KER:GR
Keratella hiemalis This organism has been called K.hiemalis due to spines are less than a third of the body length a been many specimens that are merely empty lor facet patterns. I am fairly certain that this organ reasons. I have never seen a single posterior spi K.testudo. Also the spines are always <1/2 the Until I can confirm the facet patterns I am not	nd are straight. However, there have not cicas from which we can make out the distinct nism is K.hiemalis for a number of other ined form which would indicate body length, arguing against K. quadrata.	CEW	G,I.,W	I.W6/7/88 G1/27/90	RO:KER:HI

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
	anteriomedial spines (60-70 um). There are two ace. This form does not display posterior spines.	JAA	L,W	L4/30/90 W1/26/90	RO:KER:SE
Keratella sp. This is a genus of rotifers with thickened anterior spines and 0, 1, or 2 posterior spi		CEM	G,L,W	GLW6/7/886/20/89	RO:KER
Keratella tauroce phala This species is very similar to K.cochlearis difference is that the antero-lateral spines bull horns.	Stemberger 1979 i, although slightly larger. The key is are enlongated and bowed. Thus they resemble	CEM	G,L,W	GLW6/7/88	RO:KER:TA
<i>Lecane cre pida</i> So far this species has only been recorded	Stemberger 1979 I on 9/21/91.	EMNJAA	W	W9/21/91	RO:LEC:CR
There are a pair of small cusps (not devel	Stemberger 1979 or margin of the dorsal plate is slightly convex. oped spines) on the anterolateral margin. The ps (see Stemberger). The toes are shorter than	JAA	L	L9/23/89	RO:LEC:FL
	Stemberger 1979 of the posterior margin of the ventral plate. of the organism. It has short toes (<30 um) and	JAA	G	G11/13/89	RO:LEC:LI
concave. The toes of this species are elon	Stemberger 1979 nm). The anterior margin of the dorsal plate is gated (50-60 um) and end in a sharp claw. newhat circular, more so than seen in other	JAA	G,W	G8/11/90 W8/28/89	RO:LEC:LU

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
	Stemberger 1979 e to L.luna. The anterolateral margin ends with a feature is that the foot segment extends below the	JAA	G	G7/19/90	RO:LEC:MI
is straight (compared to L.mira) with a	Stemberger 1979 130 um). The anterior margin of the dorsal plate a pair of pronounced cusps. The lorica is elliptical in tern of ridges. The toes are elongated (> 55 um) and	JAA	I.	1.8/13/89	RO:LEC:SI
The members of this genus have not be have been a number of species record	Stemberger 1979 ped foricas and display a pair of o be closely related to the Monostyla spp. been abundant in our routine samples, but there ed from the three lakes. This genus has quently and is never as common as in the other two lakes	JAA i	G,L,W	G10/9/89 I.7/6/88 W8/28/89	RO:LEC
does not have cusps. The lorica of this	Stemberger 1979 imilar to L.flexilis. The anterolateral margin s species appears to be well developed. The two her. It may be confused with a Monostyla species.	JAA	G,L	G8/11/90 L11/19/90	RO:LEC:TE
Le padella sp. This species was only seen in Lake La thus did not show up on the datasheet	Stemberger 1979 cawac. It did not fall within the counted transect and ts.	JAA	I.		RO:LEP
V-shaped. The anterodorsal notch is s	Chengalath 1976 e is strongly convex; the anteroventral notch is deep and shallow. The foot groove is parallel-sided and the edges of the last foot segment is longer than the rest.		W	W4/14/91	` RO:LEP:PA

SPECIES NAME TAX COMMENTS (J.A. Aufderheide)		PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
Le padella tri ptera Che. The lorica of this small Lepadella species (70 um) is sinus is shallow and U-shaped. The dorsal plate is ke triradiate.	almost spherical in shape. The ventral	JAA	W	W4/14/91	RO:LEP:TR
Lo phocharis sp. Sten Only one specimen of this species has been recorded with a Lepadella or Ploesoma. Since no drawings we be confident this species is truly present within Lake try to isolate this species from the L8/14/89 sample to	ere done it is difficult to e Lacawac. I would like to go and	JAA	L	L8/14/89	RO:LOP
Monommata sp. Edm This is a soft bodied species that is believed to exist The key taxonomic feature is their pair of elongated presence of this species in our samples, particularly is strong horizontal mixing of littoral waters into the p	primarily in the littoral zone. d (> 170 um) and uneven toes. The in Giles 9/24/89, may be due to	JAA	G,L	G9/24/89 L6/6/89	RO:MNO
Monostyla closterocerca Sten This is a small bodied Monostyla species (VPL > 80 concave and narrower than 3/4 of the ventral plate w below the posterior of the body. The toe (35 um) do	um). The anterior margin is slightly width. The foot segment does not extend	JAA	L	L8/2/90	RO:MON:CI
Monostyla copeis This is another small bodied Monostyla (VPL > 100 wider than 3/4 of the ventral plate width. The foot so posterior of the body. The toe (40 um) is larger in the	segment does not extend below the	JAA	G,L	G10/9/89 L12/13/90	RO:MON:CC
Monostyla lunaris This is a large bodied Monostyla (VPL > 110 um). The anterior margin is concave (see Lecane luna), b has a claw (TL 60 um). This species has not been cer this species along with M.copeis likely account for t	out without pronounced cusps. The toe crtainly recorded from Lake Giles,	JAA	G?,L	G?8/15/89 I.10/19/90	RO:MON:LL

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
classification which separates these genera. I	Stemberger 1979 by many authors. However, I am following Stember Monostyla species are loricated and similar ral body shape. Monostyla species all display a	JAA rger's	G,L,W	GL6/13/88 W6/7/88	RO:MON
AT / /					
Notholca acuminata This species is much longer than wide (BL > There is a short (<50 um) truncated posteric	Stemberger 1979 >200 um, BW <100 um). Its forica is heavily ridged or extension of the forica.	JAA	W 	W3/25/90	RO:NOT:AC
N I I.	-	•	L,W	1,12/27/893/25/90	RO:NOT
		JAA s	***	W1/27/903/25/90	
Notholca squamula	Stemberger 1979	JAA	l.	L3/25/90	RO:NOT:SQ
The lorica of this species is rounded and has <140 um and BW is <100 um. The anterola	s varying degrees of ridging patterns. The BL is ateral and anteromedian spines are of similar e much shorter. There is no posterior extension				
Ploesoma s p.	Stemberger 1979	JAA	G,L,W	G11/13/89	RO:PLO
This is a heavy loricated species. The body is	s > 100 um in length (BL) and > 70 um in width (B) of the ventral surface and are >50 um in length. Th	BW).	G,L, #	1.6/28/88 W8/2/88	ROALO
	Stemberger 1979 ica. The lorica displays a well defined pattern. and without a spinelike projection. The BL is >110	JAA	I_,W	L.6/7/90 W8/28/89	RO:PLO:TR

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
extend well beyond the posterior of the boa small pair of ventral fins, which distinguis	shes it from other large Polyarthra species (see as only eight nucleii. This species was only recorded	JAA is	W	W6/6/90	RO:POL:DO
species. This species does not display a pair dolichoptera. The swim fins do not extend	Stemberger 1979 In the distinguished from other Polyarthra In of ventral fins and so is distinguished from P. In the beyond the posterior of the body (if so not by much). In the body (FW > 50 um). The vitellarium contains	JAA	W	W8/1/89	RO:POL:EU
Pol yarthra lg. (large) This is an ad hoc grouping of what could b than 120 um. I want to distinguish this grou small adults. This group likely includes seve dolichoptera.	ip of large adults (w/eggs) from a group of	JAA	G,l.,W	GL6/19/89 W6/6/88	RO:POL:LG
This is a small species (adults with eggs are	Stemberger 1979 date. I am hesitant to change P.remata may make up the organisms in P. small. <120 um). There are four nuclei within their 25 um wide) that extend slightly beyond the body.	JAA	W	W6/6/90	RO:POL:RE
Polyarthra sm. (small) This species is designated as small (sm.) du not taken this group of rotifers out to its or of identifying them within the counting chadifferent species of the same size in this ca of the year.	onstituent species due to the difficulty amber. It is my feeling that there may be	JAA	L,W	L6/19/89 W6/7/88	RO:POL:SM

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
display cuticular swimming fins, which ventrolateral sections near the anter	Stemberger 1979 lat are cylindrical or rectangular in shape. They all the share attached in four groups to dorsolateral and lior end of the organism. The key taxonomic features larium, the length and width of the swimming fins, and body.	CEW	G,L	G6/7/886/5/90 1.6/7/886/19/90	RO:POL
fins extend slightly beyond the poste present within the vitellarium. The s	Stemberger 1979 ventral fins. The BL is > 120 um and BW > 90 um. The sw rior of the body (FL >80 um). There are eight nucleii horter swim fins and the higher body length:width P. dolichoptera. I believe that this species ifers classified as Polyarthra Ig.	JAA im	W	W8/28/89 and W6/6/90	RO:POL:VU
cross-sectional view. There is no foo thread of secreted material (Ruttner with the lorica. This species was four	Stemberger 1979 2 < 100 um). The lorica has four lobes when seen in a present. The eggs are attached to a retractile record to the contact and in high abundance in a small pond (Cook's Pond) above a downstream during periods of rain.	JAA	W	W6/19/89	RO:POM
been critically identified. CEW has s	pecies that is found in the core lakes. It has not uggested that this abundant ciliate resembles Rhabdostyla. I confounds identification. It is yellow brown in color ronucleus is a prominent feature.	CEW	L,W	LW6/7/88	CERHA
with regard to their transparent bod or live length). They contract at dead display a prominent eye-spot and the There may be more than a single spo	Stemberger 1979 oft-bodied species. They are similar to Asplanchna ies. They may be between 200-600 um in length (non-contra th, distorting the key taxonomic features. They as could be confused with G.hyptopus (see G.hyptopus). ecies that is categorized as Synchaeta sp. The likely a. Until I can look at them while still alive and look guish species.	CEW	G,L,W	GLW6/7/88	RO:SYN

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	Stemberger 1979 erminal, The lorica appears smooth. The lateral antennace body. The BL is > 120 um, The frontal eye opening.	JAA :	L	L10/19/90	RO:TES:PA
are located on the anterior third of the	Stemberger 1979 subterminal. The lateral antennae of this species lorica. The frontal eye spots are discrete. al view; thus it appears to be ridged. The BL is	JAA	L	L8/2/90	RO:TES:RE
in size and shape to Ascomorpha ovalis. The loricas are quite thin and thus are t	Stemberger 1979 are dorsoventrally flattened. They are similar However, this genus lacks the dark fecal bodies, ransparent. There have been two species identified, ontal eye spots located beneath the oral opening.	JAA	G,L	GL6/7/888/2/90	RO:TES
	Stemberger 1979 elongated (BL >280 um). There is a hooked anterior inent eye spot. There appears to be a single	JAA	L,W	L5/18/90 W10/08/89	RO:TRI:CY
This species lacks defined mucrones sur	Stemberger 1979 >170 um). It is somewhat smaller then T.cylindrica. rounding its oral cavity. They have two toes of unequal proximately 5X the length of the shorter toe. bot.	JAA	W	W1/19/91	RO:TRI:LO
much smaller species. This classification	Stemberger 1979 clarge species of Trichocerca from obvious n lasted until I was confident in my identification ely species within this group are T.cylindrica and T.multi	JAA crinis.	L,W	L6/6/895/18/90 W6/7/8810/8/89	RO:TRI:LG

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	Stemberger 1979 stiguished from others based on its ovoid shape. oximately half as wide. This species also appears rter toe is barely noticeable (TL <7 um).	JAA	L,W	L6/6/89 W6/19/89	RO:TRI:MU
	Stemberger 1979 BL 175 um), but is much thicker. Its body is /4 the length of the longer toe (long TL 50 um). nding the oral cavity.	JAA	L	I.2/24/91	RO:TRI:PO
(>130 um.). It is only slightly larger than T	Stemberger 1979 eleti, T. pusilla is a short and stout bodied rotifer rousseleti. However, it is the toe length that rousseleti is shorter (30 um) than that of T. nine mucrones of T. rousseleti.	JAA	L,W	LW1/1/92	RO:TREPU
Trichocerca rousseleti This is a small bodied Trichocerca (BL >1) margin. These mucrones are folds and not	Stemberger 1979 10 um). There are nine mucrones on its anterior spinelike. The longest toe is >30 um.	JAA	L,W	I.W6/6/90	RO:TRI:RO
		JAA	G,L,W	G7/3/90 L4/30/90 W12/27/89	RO:TRI:SI
Trichocerca sm. (small) This classification distinguishes obviously s (large)). The most likely species classified s	Stemberger 1979 mall species from larger ones (see T.Ig within this group are T.similis and T.rousseleti.	JAA	I,W	L6/6/894/30/90 W6/7/8812/27/89	RO:TRI:SM

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	I.AKES AND DATES RECOGNIZED	SPECIES CODE
these lakes all have toes of unequal len or semi-loricas. Many forms display a h	Stemberger 1979 eneous with many distinctive species. The species in 19th. The organisms within this group have soft 19th this in the lorica. Many of the species have 19th the species have 19th the species have 19th the species of the species have 19th the species of the species of the species have 19th the species of t	JAA	G,L	GL6/7/88GL6/6/89	RO:TRI
spines coming from the first joint of th body length. One specimen was positive	Edmondson 1959 onomic feature is the presence of heavy dorsal e foot. The toes are approximately 1/3 of the ely identified in Lake Waynewood. I have sketched anot . This genus is said to be primarily littoral in habit.	JAA ther	W	W7/2/90	RO:TRT
leg has only one segment. It has a singl	Edmondson 1959 000 um). They have 12 antennial segments. The fifth the inner spine and two outer setae. The inner spine of as the terminal segment. This species was opepods.	JAA	L,W	L11/11/89 W8/1/89	CY:TRO:PR
Unknown(unidentified) cili I have made little attempt to classify cil belief that there are many ciliate gener	liates even to the genus level. It is my	JAA	G,L,W	GLW 6/7/88	CI:SPP
Wolga sp. This species has a faceted dorsal surfaction on this species was	Edmondson 1959 ce. The lateral antennae lie under a spinelike projection. found.	JAA	W	W1/26/90 only	RO:WOL

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The antennules of this species are larger are nearly parallel to each other and of than 500 um. Due to their small size, the made to distinguish between adults an	Edmondson 1959 Iten oval or rounded, the carapace valves cover the body ge (compared to body size) and fixed to the carapace. The urve towards the body. The body is typically smaller hey should be counted in the 48 um, samples. An attemp d juveniles by counting the ones with eggs as Bosmina (I reladocera (Chydorus) was first started 9/10/89.	ey I was	L,W	LW7/19/88	CL:BOS
The head is small and posteriorly depr The antennules are small and fixed. T	Pennak 1978 by have oval or round valves ending with a short spine. bessed or flattened. The eye nearly fills the head vertex. there is no postanal extension of the postabdomen. The been taken to species. They appear infrequently in the	GG	W	W8/28/89	CL:CER
more than four times longer than they spike between the 1st and 2nd mandib	Cook 1956 only a single segment, the prelabral appendages are vare broad. They are leaf-like in shape. There is a small vular teeth. The antenna has a seta 1/4 to 1/3 the dibular fan has 10-12 setae. This is a large (up to 15mm) in the late instars.	JAA species	L,W	I.W6/6/89	DECHA:FL
than they are wide. There isn't a small	Cook 1956 (<12mm). The prelabral appendages are > 15 times lor spike between any of the three mandibular teeth. the middle of the antenna and is between 21-26 um in le	•	G,L,W	GLW6/7/88	DI:CHA:PU
spines. There is a pair of darkly colore	Cook 1956 rae. Their antennae are prehensile with strong apical and hydrostatic organs located in the thoracic and species present in the core lakes are C.flavicans and	CEW,JAA	G,L,W	GLW6/7/88	DECHA

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come together to form a beak-like r	Edmondson 1959 It is counted in the 202 um samples. The fornices of the cara ostrum. This rostrum partially covers the anntenules. This be, tend to be more ovoid. It is uncommon for us to find open-water samples.	AY,JAA pace	G,L,W	GLW6/7/88	CL:CHY
segments are laterally extended into as long as it is broad. The outer late This seta is approximately parallel to	Edmondson 1959 pepod (females <1.4mm). The fourth and fifth metasomal pointed wings. The caudal ramus is at least four times trail seta is 1/4 the distance from the terminus of the ramus, to the outer terminal seta of the caudal ramus. There dithe ramus is heavily ridged. The fifth leg is made up of two solds the length of the other seta.	CEW	G,L,W	GL6/7/88 W4/29/90	CY:CYC:SC
present in both the mature and imm hair arising from the base of the sec	Brooks 1957 ventral valve surface is rounded. A toothed crest may be nature forms. An ocellus is present beneath the eye. The switcond joint of the three-jointed ramus of the antenna is the sa All of the teeth of the three pectins of the postabdominal cl	ime	L	L7/7/90	CL:DAP:AM
the valve length. The interspinule d an ocellus present. The optic vesicle	Brooks 1957 d and ovoid in shape (1-2mm). The spine is less than 1/2 of istance is twice as great as the spinule length. There is a separate from the head margin. The teeth of the middle re stout and greater than three times as long as the teeth	JAA	G,L	GL7/7/90	CL:DAP:CA
larger (1-2mm). The posterior spine present. The optic vesicle is separat of the postabdomen is approximate.	Brooks 1957 If, similar to that of D. ambigua. However, D. rosea is much is thin and less than 1/2 of the valve length. An ocellus is the from the margin of the head. The second abdominal procesty 1/2 the length of the first abdominal process. All of the ual length. Nockenzahne may be present in the	JAA :ss	L	L7/7/90	CL:DAP:RC

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
Their carapace consists of two valves present on the posterioventral surface rostrum. The antennules are small and	Edmondson 1959 0.75-2.2mm) organisms with oval or elliptical bodies. that close dorsally with a posterior spine. Spinules are of the valves. The females display a distinct and pointe I fixed. The males have large moveable antennules, each have not been rigorously identified for the regular countion.	h with	G,L,W	GI.W6/7/88	CL:DAP
to calculate an egg ratio value we wou Since we are only counting adults with	mate the number of adults. We felt that in order old need to eliminate the juveniles from this calculation, a eggs we would expect our egg ratio to be an overestim the other cladocerans and they will not be included		G,L,W	GLW9/10/89	CL:DAP
segments. The caudal ramus lacks hair broad. There are two outer lateral set. The shorter seta is 1/3 the distance from The inner spine of the terminal segme outer terminal spine of the endopod of This species dominates the cyclopoids	Edmondson 1959 and 1.1 mm length. The first antenna is made up of 17 along its inner margin and is five times longer than it is ae. The longer one is 2/3 the distance from the base, om the base. The fifth leg is made up of two segments, and is <1/3 the length of the terminal seta. The if the fourth leg is two times the length of the inner spir from Nov.'89 to March '90, not M.edax as originally cold as Cyclops bicuspidatus before adopting the present n	ne. unted.	W	W10/19/90	CY:DIA:TH
The antennae are large and thickened three joints. This organism does not h ocellus present, the antennules are sm	Pennak 1978 pace that covers the mouth and legs (usual form). The dorsal ramus has two joints and the ventral ramus ave a rostrum or fornices. The eye is large and there is all and truncated. The olfactory setae are terminal, bdomen. Organisms of this genus have not been formal	no	G,L,W	GLW9/24/89	CL:DIA

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
length of exopod 1. There are only two leg is 1/2 the distance from the base to t	Edmondson 1959 5 <= 1.1mm). The female fifth leg endopod is <1/2 the setae on exopod 2. The lateral spine of the male right the terminus of exopod 2. The right endopod is <1/4 of left fifth leg there is a stout pointed process, which stal process.	ifth	G,L	GL6/7/88	CA:DIA:MI
terminal claw of the right fifth leg. The length of exopod 1. The inner process o	Edmondson 1959 ength. The male left fifth leg extends to the base of the length of exopod 2 of the left fifth leg is 3/4 the of exopod 2 is 1/3 the length of the distal process. There is fifth leg. The endopod extends down to exopod 2. The of the female fifth leg.	:	W	W6/7/88	CA:DIA:OR
well developed lobe on the inner side of pertuberance. However, there is a ventr The endopod of the female fifth leg dis There are three terminal setae on exopo	Edmondson 1959 5 < 1.6mm). The metasomal wings of the female display f both wings. The genital segment has only a slight laterally directed flange on the right side of the segment. plays two terminal setae and extends down to exopod 2 od 2 of the female fifth leg. The left endopod of the spine of the right fifth leg is 3/4 the length of	al	G	G6/7/88	CA:DIA:SP
a single segment. There is a terminal spi the first antenna are 12 segments in len the distance from the terminus of the ra	Edmondson 1959 A single male has been identified. The fifth leg consist ine (>30 um) and two long terminal setae (>70um). gth. The outer lateral seta of the caudal ramus is <1/4 amus. There is a second smaller seta located near the lateral spinules on the caudal ramus that is found with the	rger	L	L1/26/90 only	CY:EUC:AG

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERÊNCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
thin and forms a high back when seen carapace. The antennules are small ar with two joints and with annulations r	Edmondson 1959 2.5 mm), but is laterally compressed. The carapace is from the side. The mouth and legs are not covered ad fixed. The antennae of the female are uniramous sear their base. The eye is small and with many lense of fine spines. The entire organism is typically encase.	by the s, curved, es.	L,W	LW6/7/88	CL:HOL:GI
small brood sac. The antennae display and display numerous setae. The eye are six pairs of prehensile and cylindri The body consists of four segments, the are abdominal. The last segment cont	Edmondson 1959 an elongated body (7-18 mm). The carapace is reduce a heavy basal joint and have four other joints. The is large and fills the anterior end of the organism. To cally jointed legs. The first pair is longer than the one first contains the head and thorax and the remainains the stomach. The postabdomen is not reflexed cies has been described for this genus (Focke 1844)	y are very long here thers. ning three as with	G,L,W	GLW6/7/88	CL:LEP:KI
segments. There is a smooth hyaline ralong its inner margin and is twice as distance from the base. The terminal length). The fifth leg is made up of two on the terminal segment. The seta or	Edmondson 1959 tiles >1.5 mm). The first antennae is made up of 17 nembrane on the terminal segment. The caudal ram long as it is broad. The outer lateral seta is 80% of the setae are much longer than the ramus length (longer to segments. There are two long spines (>70 um) and the basal segment is the same length as the inner seta of the fourth leg endopod is > 1/3 the least not been counted in the database.	the est >7X ramus ed a median seta	L,W	W1/19/91 (JAA) L12/30/92 (PLS)	CY:MAC:AI
a sharply notched hyaline membrane. the terminal segment is longer than the long as it is broad with hairs along its	Edmondson 1959 sales >1mm). The terminal segment of the first anto the fifth leg is made up of two segments. The innest terminal seta. The caudal ramus is < four times a inner margin. The outer lateral seta is 2/3 the left to the outer terminal seta. This species is dominal and anynewood.	r spine of as	L,W	LW6/7/88	CY:MES:EE

SPECIES NAME COMMENTS (J.A. Aufderheide)	TAXONOMIC REFERENCE	PCLP INVESTIGATOR WHO IDENTIFIED THIS SPECIES	LAKES RECORDED	LAKES AND DATES RECOGNIZED	SPECIES CODE
Microcyclo ps varicans rubellus	Pennak 1989	PLS	L,W	L9/11/92 W/8/25/92	CY:MIC:VA
16 segments. The fifth leg is made up of three the exopod. There is a single long seta (>35ur is 1/3 the length. The outer lateral seta is near There is a heavy dorsal ridge, which extends fi hairs along the inner margin of the ramus. This the counters. Only one species has been described.	limnetic waters of Lake Lacawac and may have species. I have found that this species is	ner near dal ramus endicular. are	G,L,W	G3/25/90 I.7/3/91 W5/12/92	CY:ORT:MO
two terminal setae. There are four pairs of leg The antennae are stout with one ramus made The antennules are small and fixed to the veni	l process of the postabdomen is elongated and d is with subcylindrical (slightly flattened) joints. up of three joints and the ohter made up of four		G	G6/7/88	CL:POL
Tro pocyclo ps prasinus This is a small species of cyclopoid (>1000 um leg has only one segment. It has a single inner the fourth leg is less than twice as long as the originally identified as small cyclopoid copepo	spine and two outer setae. The inner spine of terminal segment. This species was	JAA	L,W	LW8/1/89	CY:TRO:PR
Unidenti fied cyclo poid The unidentified cyclopoid in Waynewood is r this suggestion on the abundance of D.t. in W	nost likely Diacyclops thomasi. I'm basing aynewood in the late summer and early fall (JA)	AMS A obs.)	W	W7/31/89	CY:UNID

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that have a Unid.cycl.(blue) in the datash	yclopoid, but a harpacticoid. From the samples neets, I have found a dark gray organism that may ganism I will not be able to positively identify it.	AMS have been blue.	L,W	LW7/3/89	
Unid. cyclo poid (small) It is very likely that this organism is T.pra		GG	w	W8/28/89	
Unid. cyclo poid (very small) It is very likely that this organism is also		KSB	W		
te is very likely that this Organism is also	T-pressines.				

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