

Regional Information Report No. 4K08-4

**Kodiak Island Lake Assessment/ Limnology Project
and Laboratory Analysis Operational Plan, 2008**

by

Steven E. Thomsen

March 2008

Alaska Department of Fish and Game

Division of Commercial Fisheries



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LABORATORY ANALYSIS OPERATIONAL PLAN**

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Steven E. Thomsen
Division of Commercial Fisheries, Kodiak

Alaska Department of Fish and Game
211 Mission Road
Kodiak, Alaska 99615

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*Steven E. Thomsen
Alaska Department of Fish and Game, Division of Commercial Fisheries,
211 Mission Road, Kodiak, AK 99615, USA*

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ABSTRACT

The Lake Assessment Project for Kodiak and Afognak Islands was started in the mid-1980s as part of a comprehensive plan to examine and prioritize the region's sockeye salmon *Oncorhynchus nerka* production potential. As part of the Kodiak Regional Comprehensive Salmon Plan, limnological and fishery investigations were initiated simultaneously to determine the appropriate enhancement and/or rehabilitation strategy for depressed sockeye salmon stocks or the stocking potential of barriered lakes without anadromous fish. The Alaska Department of Fish and Game Near Island laboratory was established in 2000 and since then has collected and analyzed limnological samples to meet these goals. This report provides the procedures used to process the limnological samples.

Key words: limnology, Kodiak Island, Afognak Island, water sample collection, zooplankton sample collection, laboratory analyses.

INTRODUCTION

Investigations of sockeye salmon *Oncorhynchus nerka* production potential in the Kodiak Island Archipelago were started prior to Alaskan statehood. Prior to statehood, the salmon resources had been heavily exploited and the salmon escapements had been severely depleted. The Alaska Territorial Department of Fisheries was created to rebuild the depleted runs and focus on expanding the sockeye salmon fisheries through enhancement techniques. Various strategies including hatchery production, stocking of barren lakes, lake enrichment, and installation of steep passes (fish ladders, fish passes) were employed to increase sockeye salmon production and harvest opportunities in the territorial days (Schrof et al. 2000). During these investigations, researchers monitored the physical, chemical, and biological parameters of the lake to maximize sockeye salmon production.

In the early 1980s, the Commissioner of the Alaska Department of Fish and Game (ADF&G) established regional planning teams (RPT), consisting of representatives from the ADF&G and the regional aquaculture associations to develop a plan for the long-term future of the salmon resources within the different regions of the state by establishing priorities and examining each region's salmon production potential (KRPT 1992). As part of this Kodiak Regional Comprehensive Salmon Plan (KRCSP), limnological data were collected along with fisheries data to assess a lake's primary and secondary production relative to juvenile sockeye salmon production.

The ADF&G began sampling Kodiak Island lakes for limnological data in the mid-1980s (Schrof et al. 2000). Lake sampling and laboratory processing of samples were difficult to sustain by the early 1990s due to the reduction of the ADF&G budget. As a result, in 1991 the Kodiak Regional Aquaculture Association (KRAA) began funding the majority of limnology sampling and laboratory analysis in the Kodiak area. The limnology program supports the long-term goals of the KRCSP and has become an integral part of salmon enhancement, restoration, and biological monitoring projects within the Kodiak Management area (Honnold et al. 1996; KRPT 1992). Over 30 Kodiak Island Archipelago lakes have been sampled for limnological data since this cooperative ADF&G/KRAA program was initiated. The limnology program is scheduled to continue in conjunction with salmon stocking and other enhancement/rehabilitation projects. The Kodiak Island Archipelago sampling project goals, objectives, and methods are published annually in an operational plan (ADF&G 2007).

Prior to 2000, water and zooplankton samples were collected by the ADF&G staff in Kodiak, processed and then shipped to the ADF&G, Division of Commercial Fisheries Central Region

Limnology Laboratory (CRL) for analysis. In 2000, the ADF&G CRL staff discontinued providing contract laboratory services for the Kodiak area. To continue the limnology program in the Kodiak area, the ADF&G started a laboratory at the Near Island Research facility (Near Island Laboratory, NIL) for the collection, processing, and analyses of water samples.

The NIL analyzes limnological samples from projects other than the ADF&G/KRAA limnology program. Afognak Lake is monitored as part of the U.S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Resource Monitoring (FRM) Program (project 04-412; Honnold and Schrof 2004). The FRM project goals, objectives, and methods are comprehensively described in the specific project operational plan (ADF&G 2007). Zooplankton samples from eight lakes located in Southeast Alaska are also processed and the data summarized at the NIL. The NIL has been processing zooplankton samples sent from the ADF&G, Region I, Division of Commercial Fisheries for the past three years. Zooplankton and nutrient samples from Salmon Lake (located near Nome) are processed and the data summarized at the NIL. The NIL has been processing nutrient samples sent from the ADF&G Region III, Division of Commercial Fisheries for the past two years. Beginning in 2007, the NIL began processing zooplankton samples sent from the ADF&G Region II, Division of Commercial Fisheries.

The NIL processes and analyzes water samples for the following nutrients and algal pigment concentrations: Total phosphorous (TP), total filterable phosphorous (TFP), filterable reactive phosphorous (FRP), total ammonia (TA), nitrate + nitrite, chlorophyll *a*, and phaeophytin *a*. Other nutrients that may be analyzed include reactive silicone and organic carbon. In addition, water chemistry parameters, such as pH and alkalinity are analyzed. Turbidity, color, calcium, magnesium, and iron may also be assessed. Zooplankton samples are processed and analyzed for species abundance, biomass, and size.

Until 2000, the Central Region Laboratory, analyzed water samples using the methods described in Koenings et al. (1987). Laboratory analysis in Kodiak at the NIL has continued to follow Koenings et al. (1987) methods; however, several procedures have been modified to reflect new techniques and utilize available laboratory equipment. Beginning in 2003, Total Kjeldahl nitrogen (TKN) analysis has been contracted out to either South Dakota University or the Central Region Laboratory. In 2003, the NIL restructured water sample processing procedures to increase production capacity. Prior processing procedures are described in ADF&G (2002).

GOALS

1. Provide sampling logistics and laboratory support for limnology data collection in the Kodiak area and assist ADF&G programs in other regions with their sample processing needs if capacity allows.
2. Assess the primary and secondary production of selected sockeye salmon nursery lakes in the Kodiak Archipelago.
3. Monitor rearing habitat of selected salmon systems to assist in lake management to maintain productive juvenile rearing and subsequent adult production.

OBJECTIVES

1. Measure water chemistry, nutrient, and chlorophyll-*a* concentration from samples collected to estimate the seasonal mean water chemistry, nutrient, and chlorophyll-*a* concentration by unit volume.
2. Quantify and measure each species or genus of macrozooplankton from samples collected to estimate the seasonal mean density, biomass, and size of each of the species or genus.
3. Collect light attenuation data to estimate the euphotic zone depth (EZD) for algal photosynthesis.
4. Determine the temperature and dissolved oxygen regimes.

TASKS

A detailed limnology program lake sampling schedule with a lake location map is included as an attached memorandum as part of the Salmon research operational plans for the Kodiak area (ADF&G 2007). This memorandum details yearly changes in the limnology sampling program and lists specific lakes, stations, and depths to be sampled. The following is a generalized list of NIL tasks.

At each established limnology station:

1. Collect lake water.
2. Collect zooplankton.
3. Collect depth profiles of light attenuation (Foot Candles).
4. Measure depth profiles of dissolved oxygen (mg/L) and temperature (°C).
5. Measure the water clarity.
6. Process and analyze the above mentioned water and zooplankton samples.
7. Process and analyze water and/or zooplankton samples from any additional subcontracts.
8. Enter and archive all data into established formats. All data collected by the ADF&G are available to the public upon specific request.
9. Schedule field sampling with approved air charters companies.
10. Adhere to the department's Standard Operating Procedures (SOP), with emphasis on laboratory, aircraft, and boating safety.

SAMPLE COLLECTION PROCEDURES

PRIOR TO DEPARTURE

1. Assemble all the required sample collection gear from the *Field Sampling Equipment List* (Appendix A1).
2. Consult the sampling schedule in the yearly memorandum to verify which lakes, stations, and depths are to be sampled.

3. Obtain a clearly marked morphometric map showing global positioning system (GPS) station locations by latitude and longitude at the lake(s) being sampled. Maps are on file in the Kodiak office and at the NIL.
4. Clean the 8 L plastic carboy's needed for the sampling trip. The carboys are pre-labeled with the name of the lake, station, and depth.
5. Clean and label the 125 ml polypropylene zooplankton bottles needed for the sampling trip.
 - a. Label the bottles with the name of the lake and station. The date and tow depth will be recorded at the time of sampling.
 - b. Add 12.5 ml buffered formalin (10% of volume) to each polypropylene bottle, replace the cap and seal the lid with electrical tape to prevent the contents from leaking out.

LAKE STATION SET-UP

1. Locate the appropriate lake station location using a GPS and lake map.
2. Set the lake station buoy. Typically, lake stations are left in place over winter. Winter ice tends to move the stations requiring them to be reset to the proper location. If a station is missing, set a new buoy using the following procedures:
 - a. Fill three sand bags on the lake shore.
 - b. Attach the sand bags to the end of the spool of ¼" polypropylene rope.
 - c. Allow the sand bags and rope to sink to the lake bottom. A pipe placed through the spool of polypropylene rope is recommended.
 - d. Attach a buoy to the lake station line. Leave a few feet of scope in the line to prevent dragging the buoy.

WATER SAMPLING

1. Collect water samples at designated lake stations using a Van Dorn sampler.
 - a. Lower the Van Dorn sampler to the designated depth (usually, 1 or 50 m).
 - b. Attach a weighted messenger to the rope. Drop the messenger down the rope to release the mechanism that triggers the closing of the Van Dorn sampler.
 - c. Pull the Van Dorn sampler to the surface. Rinse the pre-labeled carboy with a small portion of sample water and discard the rinse water. Rinsing removes any DI water left in the carboy. Pour the remaining sample water into the carboy.
 - d. Repeat this procedure, if needed, (without rinsing) until the carboy contains a minimum of 3.8 liters of sample water. Any changes in the volume of sample water required will be listed in the yearly memorandum. When finished, place the carboy in the airplane floats or a cooler to keep the sample cool and dark.
 - e. Record sampling depths, stations, and other appropriate comments on the *Detailed Lake Survey (DLS) Form* (Figure 1).

ZOOPLANKTON SAMPLING

1. Collect zooplankton samples at designated stations using the vertical tow method, using a 0.2-m diameter, 153-micrometer mesh conical net.
 - a. Measure the station depth by lowering a weighted, metered line to the lake bottom. This step is unnecessary for stations with a depth greater than 51 meters (refer to step b).
 - b. Lower the tow net into the lake at a steady rate (so that the plankton bucket stays below the opening of the net) until the bottom of the net is about 1 meter from the lake bottom or to a maximum of 50 meters.
 - c. Retrieve the net manually at a constant rate of approximately 0.5 m/sec., stopping with the rim of the net just above the surface, slowly working the contents of the tow towards the plankton bucket.
 - d. Rinse the net from the outside with filtered water from a squirt bottle from the top down to the cod-end to wash the contents into the plankton bucket.
 - e. Remove the plankton bucket from the net and pour the contents into the appropriately labeled 125-ml polypropylene bottle.
 - f. Repeatedly, rinse the plankton bucket screen with the squirt bottle containing filtered water and pour the contents into the polypropylene bottle. Repeat until the plankton bucket screen is clean and the zooplankton sample bottle is full.
 - g. Record the tow depth on the DLS form.

LIGHT ATTENUATION

Use an electronic photometer to measure light attenuation at the designated limnology stations. The Protomatic photometer we use is capable of measuring both "up" and "down" measurements. Only "up" (sensor oriented towards the surface) measurements will be conducted and recorded.

A consistent amount of light is needed for the duration of the measurements to provide reliable data. Measurements need to be taken on the sunny side of the plane or boat to avoid shading the readings. If cloud cover varies intensely, light intensity readings will not decrease with depth as expected. When measurements fluctuate wildly, wait for the cloud cover to stabilize and start again.

1. Record light readings (foot candles) beginning just above the lake surface (incidence), then just below the surface and at 0.5 m intervals to a depth of 5 m (i.e., 0.5, 1, 1.5, ..., 5) and then take measurements every meter (5, 6, 7, etc.) thereafter until the light level is 1% of the incidence reading.
2. Record the data on the DLS form (Figure 1).

TEMPERATURE AND DISSOLVED OXYGEN MEASUREMENTS

1. Measure water temperature ($^{\circ}\text{C}$) and dissolved oxygen (DO; mg/L) levels at designated lake stations with a YSI 52B dissolved oxygen meter.

- a. Examine the membrane on the meter probe prior to use for air bubbles and condition (e.g., tears, folds) and replace if necessary.
- b. Turn the meter on for 5 to 10 minutes with the probe in the water to warm up and then calibrate as described on the back of the meter.
- c. Measure the surface temperature with a handheld thermometer and compare to the meter temperature to ensure that the meter is working properly.
- d. Verify the DO reading by taking the surface water temperature and referring to the chart on the back of the meter. Verify the DO reading at each lake.
- e. Measure DO readings from the surface to 25 m at 1 m intervals and then at 5 m intervals thereafter to the bottom. During isothermic periods (spring and fall), readings will be taken at 5 to 10 m intervals.
- f. Record the data on the DLS form (Figure 1). The pilot of the airplane usually helps with the recording duties.

SECCHI DISK MEASUREMENT

1. Record Secchi Disk (SD) measurements at designated stations as a measure of water clarity. SD depth should be measured on the shaded side of the boat or airplane. From an airplane, measure the SD from under the wing. Remove Polaroid glasses before measuring SD depth.
 - a. Lower the SD into the water on a metered line until it disappears from view; then pull upward until it reappears.
 - b. Record the depths of disappearance, reappearance, and average of the two readings on the DLS form (Figure 1).

GENERAL LABORATORY PROCEDURES

SAFETY CONSIDERATIONS

Many chemicals used in the laboratory require special handling procedures. The laboratory has all the necessary safety equipment (i.e. hoods, eye wash stations, shower, fire extinguisher, eye protection, and personal protective clothing) to comply with safety regulations. Laboratory personnel need to recognize health and safety hazards.

1. Read the departments SOP on laboratory safety. The reference, Standard Methods for the Examination of Water and Wastewater, 20th Edition (1998) has a wealth of information on safety, chemical use, chemical and biological testing methods, quality control, and much more. Relevant SOPs are on file at the lab.
2. Follow material safety data sheet (MSDS) procedures. MSDS sheets are on file at the laboratory for reference and describe special procedures required for the handling, storage, and use of specific chemicals. All chemicals must be stored according to the MSDS sheets.
3. Most chemicals require the use of a hood and gloves. Consult the MSDS for proper procedures prior to handling chemicals.

4. Limit most reagent use and chemical disposal to the hood (including cuvette waste). Refer to each *Sample Analysis Procedures* section for specifics.
5. Always add acids or bases to water. Never add water to acids.

DE-IONIZED (DI) WATER PREPARATION

The NIL has DI filtered water available through a faucet at the sink. This DI water is filtered on the other side of the building and could pick up contamination in its passage through the extensive pipes. Re-filtering the supplied DI water ensures proper quality. The tap water can be filtered but this decreases the life of the filter cartridges. Prepare DI water with a Barnstead B-Pure filtration unit with ultrapure and pretreatment cartridges using the following methods:

1. Connect the left (when facing unit) hose to the DI water faucet.
2. Place the right hose in the sink, turn on the water faucet, and allow water to flow through the system for several minutes.
3. Connect the power cord. As long as readings remain above 14.0 meg-ohm, place the right hose into the DI container.
4. If the readings fall below and remain under 14.0 meg-ohm the filters need to be replaced. See the product manual for filter replacement procedures.
5. Disconnect the power cord and filtration unit when finished.

CLEANING GLASSWARE AND PLASTICS

1. Wash all glassware and plastics with phosphate-free soap and rinse with tap water four times and again four times with DI water.
2. Never use containers that have previously contained formalin or Lugols (e.g. zooplankton, phytoplankton, or smolt sample bottles) for water samples.

ACID WASHING

1. Use 10% Hydrochloric acid (HCl) to clean all glassware used for TA, TP, TFP, and FRP testing.
To mix 10% HCl add 100 mL concentrated HCl to 800 mL DI water, mix, cool, and dilute to 1 L with DI water.
2. Acid wash glassware before each use, keeping covered with appropriate caps at all times. Always acid wash all glassware in the fume hood and dispose of waste into a waste container in the fume hood.
3. Do not acid wash glassware used for Chlorophyll *a* analysis. A small amount of weak acid (0.05 ml of 2 N HCL) is used to differentiate chlorophyll *a* from phaeophytin *a*.

THAWING WATER SAMPLES

Freezing water samples is required if they are not to be processed within 3 days after collection. Slowly thaw the water samples in a water bath at no more than 20 °C or in the refrigerator before use. Overheating the sample can break chemical bonds. Always completely thaw the sample. If

possible analyze the total ammonia (TA) concentration first because it changes rapidly with repeated freezing and thawing.

REAGENT MIXING

Maintain a reagent mixing log. The log is posted on the main fume hood in room 205. Include the date, type, and volume of reagent mixed. This log is required by the city of Kodiak, state, and federal agencies in conjunction with a chemical disposal log (covered below). Mixed reagents degrade with time, changing the accuracy of the nutrient tests (for an example; Table 2 and Figure 3). Tracking the age of reagents helps to maintain quality laboratory data.

Sometimes the volume of a reagent to be prepared for a test will need to be adjusted when the number of samples to be run changes. When changing reagent volumes alter all added ingredients by the same proportion. For example, if you have an ending volume of 500 mL (i.e. the reagent is diluted into a 500 mL volumetric flask) and you are running 50% of the normal samples, cut all added ingredients by 50% and dilute the reagent into a 250 mL volumetric flask. The proportions listed in the test sections are appropriate for the current laboratory production level.

REAGENT DISPOSAL

Maintain a chemical disposal log; laboratory procedures produce hazardous waste material. Logs are posted on all fume hoods. Include the date, type, and volume of all types of chemicals disposed of into the fume hood or sink drains. Each *Sample Analysis Procedure* section contains specifics on chemical handling and dumping. Consult MSDS sheets for specifics. The logs must be maintained to adhere to the city of Kodiak, state (ADF&G and DEC), and federal agency (OSHA and EPA) regulations.

PREPARING STANDARDS

Standards are used to produce serial dilutions (known concentrations) for processing water samples (Tables 1, 3, 4, 5, 6, and 7). Each nutrient test includes a table giving specific serial dilutions to prepare. Accuracy and cleanliness are extremely important in preparing sample standards.

1. Mix the standard in a volumetric flask of the correct capacity.
2. Use weighing funnels to reduce chemical spillage. Pour the weighing funnel contents into the appropriate volumetric flask and rinse the funnel into the volumetric flask with DI water or the appropriate dilution agent.
3. Choose a pipette with the appropriate capacity.
4. Serial dilutions need to be made on the same day a test is run.

CUVETTE USAGE

Cuvettes are small, clear, sample containers, which are placed in the spectrophotometer to measure absorbance. Standard rectangular (10 mm light path) cuvettes are used in tests with high concentrations of nutrients and cylindrical long path (100 mm light path) cuvettes are used in

tests with low concentrations of nutrients. Cuvettes are matched to each other and need careful handling to maintain accurate readings.

1. At the start of each season fill a set of cuvettes with DI water to compare absorbances at different wave lengths. Make sure they match perfectly.
2. Always wear gloves when handling cuvettes because finger prints can affect light absorbance. Special soft, lint-free cleaning materials are needed to wipe the cuvettes clean.
3. Look at a bright light through the filled cuvette to check for anything left on the surface (e.g. water spots or fingerprints) that will affect the readings.
4. After recording readings, empty the cuvette contents into a chemical disposal container under the fume hood (cuvette contains hazardous materials). Wash the cuvette thoroughly with phosphate free soap; rinse with DI water, and acid wash. Do not brush or scrub the cuvettes because they will scratch.

FORMULATING LINEAR EQUATIONS

Serial dilutions (a range of concentrations of a standard) given in each test section are measured in the spectrophotometer at the given wave length and plotted with their corresponding concentration to produce a linear equation. Water samples (unknown concentrations) are measured simultaneously in the spectrophotometer and their corresponding value is entered into the linear equation provided by the serial dilutions to produce a concentration value. Formulate a linear equation by regressing known serial dilution concentrations against averaged absorbances (spectrophotometer measurements), and calculate the coefficient of determination (r^2) using the following procedures (for example; Table 1, Figure 2):

1. The standard linear equation is $y = mx + b$; where y = the calculated concentration, m = the slope of the line (rise over run), x = the spectrophotometer measurement (in nm), and b = the y intercept.
2. Enter the serial dilution volume (mL) into column 1 and the volume (mL) of DI water or appropriate dilution agent added into column 2 in an Excel spreadsheet (Table 1). These two columns added together constitute the total volume of the sample standard. Enter the known concentration value (units vary with test type) of the serial dilutions into column 3.
3. Enter the serial dilution absorbance (spectrophotometer measurement in nm) for the appropriate wave length into column 4.
4. Subtract the “blank” serial dilution (0.0 concentration; see Table 1) absorbance value from each of the serial dilution absorbances in column 4 and put the result into column 5 (i.e. the value in the first row of column 4). This corrected absorbance subtracts the absorbance associated with the addition of reagents at a concentration of zero.
5. Plot the data using a scatter plot, setting the X values to equal the adjusted sample absorbances (column 5), and the Y values to equal the secondary standard concentrations (serial dilutions) in column 3.
6. Choose “add a trendline” to add the linear equation and r^2 value to the graph. Caution: Do not force the values through zero. Forcing the line through zero changes the y intercept (b) and in most cases gives an unsatisfactory result.

7. Calculate sample nutrient concentrations (y in the formula, $y = mx + b$) by subtracting the same averaged blank absorbance from the averaged sample nutrient absorbances, and substituting these values into the regression formula (x in the formula, $y = mx + b$) calculated by the serial dilutions.

QUALITY ASSURANCE

To ensure precise and accurate data, quality assurance measures must be taken every day. Ensuring quality assurance includes careful attention to cleanliness (e.g. glassware and cuvettes), exact measurements when preparing and adding reagents and standards, the use of standards with every nutrient batch, and the plotting of reagent ages (for example; Table 2, Figure 3). To further decrease variability between sample runs the NIL uses many reagents only the day after they were made. To check overall accuracy use blind quality control nutrient standards and send duplicate water samples to independent laboratories.

SAMPLE PROCESSING PROCEDURES

EQUIPMENT PREPARATION

Refer to Appendix A2 for equipment needed to filter water samples in the laboratory. Refer to Appendix B1 when filtering water samples with one manifold. Refer to Figure 4 when filtering water samples using two vacuum pumps and proceed as follows:

1. Wash the filtration equipment (i.e. filter towers, flasks) and graduated cylinders with phosphate-free soap, and then rinse with tap water four times and again four times with DI water prior to filtering. Cover the graduated cylinders and filter towers with parafilm to keep out contaminants.
2. Attach a vacuum pump to a three place filtration manifold and attach three filtration flasks to each hose of the manifold. See apparatus A in Figure 4. Apparatus A is used to collect one particulate filter pad (C, N, or P) and up to 1,000 mL of filtered water from each of three samples. Set pump suction at 15 psi.
3. Attach a vacuum pump to a waste collection carboy and attach a three place filtration manifold with three filtration towers to the carboy. See apparatus B in Figure 4. Apparatus B is used to collect chlorophyll *a* for each of three samples. Chlorophyll-*a* samples are always processed using the same graduated cylinders and filtration towers. Set pump suction at 15 psi.
4. Separation of the two collection procedures is used to prevent magnesium carbonate residue from contaminating equipment used for collection of the particulate filter pads (C, N, or P) and filtered water.
5. Keep the vacuum pump oil reservoir filled to the red line.

SAMPLE PRESERVATIVES

The following sample preservatives are used to stabilize chlorophyll *a*, phytoplankton, and zooplankton samples, respectively.

1. Magnesium carbonate (MgCO_3) – Add 1 g of magnesium carbonate-n-hydrate to 100 mL of DI water. Magnesium carbonate settles after sitting so shake well before adding to the chlorophyll *a* filter tower.
2. Lugol's acetate – Add 10 g of potassium iodide, 5 g of iodine, and 5 g of sodium acetate-trihydrate to 70 mL of DI water.
3. Buffered formalin – Add ~ 10 pellets of sodium hydroxide (NaOH) to one liter of concentrated formalin (37% formaldehyde) or until a pH of 7 to 8 is reached.

WATER SAMPLE PROCESSING

Immediately refrigerate water sample carboys when received at the laboratory. Samples must be frozen if they cannot be processed within three days. The water sample processing procedures outlined below follow the flow chart in Appendix A3.

Unfiltered Water Sample Procedures

Rinse and label a polypropylene bottle of appropriate type in steps 1, 2, and 3 with a small portion of sample water from each carboy to eliminate any DI water remaining in the sample bottles. Fill the following sample collection bottles directly from the sample carboy:

1. Unfiltered refrigerated sample bottle: Measure approximately 500 mL of sample water into a 500 mL polypropylene bottle (fill to the top to avoid trapped air) and refrigerate. Seal the bottle with tape to prevent leakage if transportation of the sample is needed.
2. Unfiltered frozen sample bottle: Measure about 450 mL of sample water into a 500 mL polypropylene bottle (leave space at the top to allow for expansion while freezing) and then freeze. Seal the bottle with tape to prevent leakage if transportation of the sample is needed.
3. Unfiltered phytoplankton sample bottle: (No collection scheduled for 2007). Measure 100 mL of unfiltered water and place into an amber 125 mL polypropylene bottle. Place a clean microscope slide into the polypropylene bottle to preserve diatoms by assuring silicon uptake. Add 2.0 mL Lugol's acetate, mix gently, and store in the dark at room temperature. Seal the bottle with tape to prevent leakage if transportation of the sample is needed.

Filtered Water Sample Procedures

Filtered water sample processing is grouped into two categories; collection of particulates and collection of filtered water. Particulate sample collection is further divided into collection of a filtrate pad used for chlorophyll *a* analysis and collection of a filtrate pad we freeze but do not process. The latter frozen filtrate pad can be analyzed for particulate C, N, or P. Filtered water used for analysis is collected from the filtration flask used to collect the C, N, or P filtrate pad. Filtered water cannot be collected using the chlorophyll *a* filtrate pad because magnesium carbonate is added as a preservative in the filtration process. Using the two vacuum pumps process the following:

1. Particulates:
 - a. With sterile forceps, place a sterile Whatman GF/F filter pad on each of the six filter towers.
 - b. Draw 100 mL of DI water through all three filter pads of apparatus A (filtrate collection) and discard the rinse water.

- c. Choose a water sample and rinse two graduated cylinders (previously cleaned) with approximately 200 mL of sample water and discard.
 - d. Fill the rinsed graduated cylinders with 1,000 mL of sample water and place one in front of the left filtration tower of apparatus A, position C, and the other in front of the left filtration tower of apparatus B, position C.
 - e. Repeat steps c and d for water samples two (use center position B) and three (use right position A). Position placement is important to keep track of samples.
 - f. Pour 100 mL sample water from the graduated cylinders in front of apparatus A into each of the three corresponding filter towers. Draw the water into the filtration flasks, rinse, and discard. This rinses the DI water from the filtration flasks.
 - g. Fill each of the six filtration towers with sample water from its corresponding graduated cylinder. Filter and add more sample water to each filtration tower until 950 mL is filtered from each of the samples. For apparatus A (filtrate) filter the remaining 50 mL and continue to step “i”, without rinsing. For apparatus B (chlorophyll *a*) proceed to step h.
 - h. When ~ 50 mL of the sample remains in the chlorophyll-*a* filtration towers add ~5 mL of the magnesium chloride (MgCO₃) solution to the filtration towers to preserve the sample. Wash all three filtration towers from apparatus B with DI water to ensure that all the particulates are collected on the filters. Do not wash the filtration towers from apparatus A with DI water because this would dilute the filtered water used for the filtered frozen sample.
 - i. Remove the particulate nutrient filter pads with forceps from the filter towers and place in the appropriately labeled, covered filter holder and store in the freezer.
 - j. Save the filtered water collected from apparatus A (2,000 mL filtration flask) for the filtered frozen sample. Discard the filtered water collected from apparatus B (waste carboy).
2. Filtered frozen sample bottle: Rinse the filtered frozen 500 mL polypropylene bottle with a small amount of filtrate water from the filtrate flask (from apparatus A) and discard. Pour approximately 450 mL of the filtrate water into the polypropylene bottle and store in the freezer. Seal the bottle with tape to prevent leakage if transportation of the sample is needed.

SAMPLE ANALYSIS PROCEDURES

pH

Almost every chemical reaction is pH dependent, making it an important aspect of water quality. pH units are a measurement of the concentration of hydrogen ions (acidity) in a solution at a specific temperature and are important in determining alkalinity (Koenings et al. 1987).

Equipment

The following equipment is required: Corning model 430 pH meter, thermometer, 100 mL graduated cylinder, and 250 mL beakers.

Chemical Handling and Disposal

Buffer and KCL fill solutions are mildly hazardous, requiring no special handling or disposal. Wash hands after use and avoid extended contact. Consult MSDS for specifics.

Reagents

1. Buffer solutions of pH 4 and 7 used to calibrate the pH meter. Store the pH electrode in a pH 4 or 7 buffer solution.
2. Electrode fill solution of 3 M KCl.

Procedure

1. Calibrate the pH meter according to the manufacturer's instructions (Corning instruction manual, 1996). Fill the electrode with 3 M KCl if needed.
2. Pour 100 mL of the unfiltered refrigerated water sample into a 250 mL beaker. Allow the sample to sit out on the counter and reach 25°C.

The Corning model 430 pH meter using the high performance probe does not automatically correct for temperature. The pH readings differ at different temperatures. For example; a pH 7.0 buffer tested at 0°C would read 7.12, at 25°C it would read 7.0, and at 50°C it would read 6.97.

3. Remove the rubber plug from the pH probe. Immerse the pH probe into the sample and stir briefly to clear the electrode fill solution from the probe. Record the measurement when equilibrium is reached. Stirring affects the pH so be sure to allow adequate time for the sample to stabilize.
4. Rinse the probe with DI water and blot dry.
5. Continue with the next sample or place the probe back into the electrode storage solution.

ALKALINITY

A lake's alkalinity determines its ability to resist changes in pH (Koenings et al. 1987). Many chemical reactions, such as photosynthesis, affect the pH and alkalinity (buffering capacity) and are important to the health of the lake. Alkalinity is principally due to the presence of carbonate and bicarbonate ions, which are converted to carbon dioxide at a pH of 4.5. A measure of alkalinity can determine the amount of inorganic carbon available for algal uptake.

Equipment

The following equipment is required: Metler Toledo Seven Easy pH meter, 10 mL buret, buret stand, magnetic stirrer, large magnetic stir bar, volumetric flasks, pipettes, 100 mL graduated cylinder, and 250 mL beaker.

The use of two pH meters, one for pH and one for alkalinity, decreases the time needed to stabilize water samples because the pH stabilizes near 7 and alkalinity stabilizes at 4.5. A thermometer is not needed for the Metler Toledo Seven Easy pH meter because it automatically corrects for temperature.

Chemical Handling and Disposal

Buffer and KCL fill solutions are mildly hazardous, requiring no special handling or disposal. Wash hands after use and avoid extended contact. Concentrated sulfuric acid is very reactive and a health hazard. Prevent any skin contact and handle or dispose of only in the fume hood. The dilute 0.02 N sulfuric acid is a mild irritant. Consult MSDS for specifics.

Reagents

1. 1 N sulfuric acid (H₂SO₄) – Add 27.8 mL of concentrated sulfuric acid to ~ 800 mL of DI water and dilute to 1 L with DI water. Concentrated sulfuric acid is 36 Normal (N).
2. 0.02 N sulfuric acid – Dilute 10 mL of 1 N sulfuric acid to 500 mL with DI water.
3. Buffer solutions of pH 4 and 7 to calibrate the pH meter.

Procedure

1. Calibrate the pH meter according to the manufacture's instructions (Metler Toledo instruction manual, 2003).
2. Measure 100 mL of the unfiltered refrigerated water sample into a 250 mL beaker. Place a stir bar into the 250 mL beaker and place the beaker on a magnetic stirrer. Place the pH meter probe into the beaker with the water sample.
3. Fill the 10 mL buret with 0.02 N sulfuric acid (titrant). Slowly add titrant to the water sample, stirring to mix the sample. Allow the sample to stabilize after additions, while monitoring the pH. Add titrant until a pH of 4.5 is reached and record the volume (mL) of titrant used.

Calculations

$$\text{Total Alkalinity (mg L}^{-1}\text{ as CaCO}_3\text{)} = \frac{B \times N \times 50000}{V}$$

Where:

B = mL of titrant added

N = normality of the titrant

V = sample volume in mL

Or: If the above procedure is followed:

$$\text{Total Alkalinity} = B \times 10$$

COLOR

The presence of organic compounds and colloidal particles imparts color and restricts light penetration (Wetzel and Likens. 1991). Color is one factor in light penetration and affects the photic depth (1% of surface illumination).

Equipment

The following equipment is required: Spectronic Genesys 5 spectrophotometer and 10 mm cuvettes.

Chemical Handling and Disposal

The platinum cobalt standard is mildly hazardous, requiring no special handling or disposal. Wash hands after use and avoid extended contact. Consult MSDS for specifics.

Reagents

500 platinum cobalt unit standard.

Procedure

1. Prepare thirteen serial dilutions of the cobalt standard using the dilution standards summarized in Table 3.
2. Measure the absorbance of the serial dilutions at 400 nanometers (nm) against a DI water blank in the spectrophotometer (Spectronic 1996).
3. Plot the values in Excel and run a regression to calculate a linear formula and a r^2 .
4. Measure the absorbance of duplicate filtered frozen water samples at 400 nm in the spectrophotometer against a DI water blank.

Calculations

1. Formulate a linear equation by regressing the cobalt serial dilution concentrations against their respective absorbances, and calculate the r^2 . Follow the procedures under the *Formulating Linear Equations* section.
2. Determine the water sample concentration by subtracting the average blank (0.000) serial dilution absorbance value from the average water sample absorbance value and insert the value into the regression formula provided by the serial dilutions.

TOTAL AMMONIA (TA)

Lake nitrogen includes organic nitrogen, ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^- ; Koenings et al. 1987). Phytoplankton utilize inorganic nitrogen, but prefer ammonium and ammonia. The Total Ammonia test determines ammonium and ammonia concentration.

Equipment

The following equipment is required: Spectronic Genesys 5 Spectrophotometer, 50 mL stoppered cylinders, and 100 mm cuvettes.

Chemical Handling and Disposal

The reagents used in this test present a health hazard. The phenol solution is very hazardous to the health. Special handling and disposal is needed when using these reagent solutions. To prevent inhalation of hazardous fumes rinse cuvettes, stoppered cylinders, and reagent bottles in the hood. Consult MSDS for specifics.

Reagents

1. Phenol solution – Dissolve 25.0 g of phenol ($\text{C}_6\text{H}_6\text{O}$) into ~100 mL of reagent alcohol (ethyl; $\text{C}_2\text{H}_5\text{OH}$), and dilute to 250 mL with reagent alcohol.

2. Ferrocyanide solution – Dissolve 1.55 g of potassium ferrocyanide-trihydrate ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$) into ~200 mL of DI water, and dilute to 250 mL with DI water.
3. Hypochlorite solution – Dissolve 80 g of sodium citrate-trihydrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) and 4 g of sodium hydroxide (NaOH) into ~300 mL of DI water. Add 100 mL of chlorine bleach (5% sodium hypochlorite), and dilute to 500 mL with DI water. *Caution:* most chlorine bleach is 6% sodium hypochlorite requiring only 83.3 mL. Opened sodium hypochlorite bottles have a two-month shelf life.

To decrease variability between sample runs the NIL only uses the phenol, ferrocyanide, and hypochlorite solutions on the day after they were made.

Standards

1. Primary nitrogen standard ($0.2 \text{ mg mL}^{-1} \text{ N}$) – Dissolve 0.3824 g of ammonium chloride (NH_4Cl) into ~400 mL of DI water, and dilute to 500 mL in a volumetric flask. The primary standard should be replaced after six months.
2. Secondary standard ($0.5 \text{ } \mu\text{g mL}^{-1} \text{ N}$) – Dilute 1.25 mL of the primary standard to 500 mL with DI water in a volumetric flask. The secondary standard should be replaced after three months.

Procedure

Add the three reagents in steps 3 through 5 to the stoppered cylinders (SC) in groups of three to stagger color development, allowing for proper processing time.

1. Label two 50 mL SC for each water sample, two SC for 0.0 and 5.0 serial dilutions, and one SC for each of the five remaining serial dilutions listed in Table 4. Run serial dilutions with each run when samples are processed.
2. Pour 50 mL of the filtered frozen water sample or serial dilution into an acid washed 50 mL SC. Process sample water and serial dilutions simultaneously.
3. Add 2 mL of phenol solution and invert to mix.
4. Add 2 mL of ferrocyanide solution and invert to mix.
5. Add 5 mL of the hypochlorite solution, invert twice to mix, and invert again after 15 minutes.
6. Allow exactly 2 hours for full color development. Readings are time dependent. Measure the serial dilution and water sample absorbances against a DI water blank at 640 nm.

Calculations

1. Formulate a linear equation by regressing the nitrogen serial dilution concentrations against their respective absorbencies minus the average blank (0.000) serial dilution absorbance, and calculate the r^2 . Follow the procedures under the *Formulating Linear Equations* section. Refer to Figure 5 for an example plot of the serial dilutions listed in Table 4.
2. Determine the water sample concentration by subtracting the average blank (0.000) serial dilution absorbance from the average water sample absorbance and insert the value into the regression formula provided by the serial dilutions.

NITRATE AND NITRITE

Nitrite (NO_2^-) is oxidized to Nitrate (NO_3^-) by nitrification and assimilated by phytoplankton during photosynthesis in oligotrophic systems (Koenings et al. 1987). Low oxygen concentrations in eutrophic systems can fuel denitrification ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NH}_3$). This method determines nitrate + nitrite concentrations by reducing nitrate to nitrite with cupric sulfate. Nitrite concentrations can be calculated by adding the reagents and not passing the water sample through the cadmium reduction columns, eliminating the reduction step.

Equipment

The following equipment is required: Spectronic Genesys 5 Spectrophotometer, 80 mL cadmium reduction columns (Figure 6), 50 mL stoppered cylinders, and 100 mm cuvettes (10 mm cuvettes can be used but are less accurate).

Chemical Handling and Disposal

The buffer, cupric sulfate, and cadmium solutions are very hazardous to the health. Avoid inhaling cadmium dust. All of these solutions require special handling and disposal. The cadmium waste must be stored for disposal and cannot be dumped into the hood. Consult MSDS for specifics.

Reagents

1. Buffer solution – Dissolve 100 g of ammonium chloride ($(\text{NH}_4)_2\text{CO}_3$), 20 g of sodium borate-10-hydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), and 1 g of Na-EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) in ~ 750 mL of DI water, and dilute to 1 L with DI water.
2. Sulfanilamide solution– Add 50 mL of concentrated hydrochloric acid (HCl) to ~ 150 mL of DI water then dissolve 3 g of sulfanilamide ($\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$) and dilute to 250 mL with DI water.
3. NNED solution– Dissolve 0.12 g of N-(1- naphthyl)ethylenediamine dihydrochloride ($\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCL}$) in ~50 mL of DI water, and dilute to 100 mL with DI water. Keep in a dark bottle and replace monthly or when a brown coloration develops.
4. Cupric sulfate solution– Dissolve 25 g of cupric-sulfate 5-hydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in ~400 mL of DI water and dilute to 500 mL with DI water.
5. 10% Hydrochloric acid – Slowly add 5 mL of concentrated hydrochloric acid (HCl) to ~ 40 mL of DI water and dilute to 50 mL with DI water.
6. Cadmium – 40 to 60 mesh granules.

Standards

1. Primary nitrate standard ($0.1 \text{ mg mL}^{-1} \text{ N}$) – Dissolve 0.36101 g of potassium nitrate in ~ 400 mL of DI water, and dilute to 500 mL with DI water in a volumetric flask.
2. Secondary nitrate standard ($1 \text{ } \mu\text{g mL}^{-1} \text{ N}$) – Dilute 2.5 mL of the primary standard to 250 mL with DI water in a volumetric flask.
3. Primary nitrite standard ($0.1 \text{ mg mL}^{-1} \text{ N}$) – Dissolve 0.24630 g of sodium nitrite in ~ 400 mL of DI water, and dilute to 500 mL with DI water in a volumetric flask.

- Secondary nitrite standard ($1 \mu\text{g mL}^{-1} \text{N}$) – Dilute 1.0 mL of the primary standard to 100 mL with DI water in a volumetric flask.

Procedure

Test the reduction efficiencies (RE) of each of the cadmium columns at the start of the season, at mid season, or after several months of not being used. If the RE is less than 95% or greater than 105%, clean and repack the cadmium columns as described below. If the cadmium columns are operating properly follow the *Water Sample Processing Procedure* section below.

Testing Cadmium Column Reduction Efficiencies

- Check the RE of each column with the following procedure:
 - Prepare a 0.0 serial dilution (blank) by adding 5 mL of buffer solution to 50 mL of DI water in a SC and invert to mix.
 - Prepare four nitrate standards. Add 10 mL of the secondary nitrate standard to two SC and add 5 mL to two SC, dilute each to 50 mL with DI water, and add 5 mL of buffer. Invert to mix.
 - Prepare two nitrite standards. Add 10 mL of the secondary nitrite standard to one SC and add 5 mL to one SC, dilute to 50 mL with DI water, and add 5 mL of buffer. Invert to mix.
 - Pour the 10 mL standards ($200 \mu\text{g/mL}$) through the cadmium column using the *Water Sample Processing Procedure* section below in the following order; blank, nitrate, nitrite, and nitrate.
 - Calculate the reduction efficiency of the column at $200 \mu\text{g/mL}$ as follows:
$$\text{RE} = \frac{(\text{averaged absorbencies of nitrate standards}) - \text{blank}}{(\text{absorbance of nitrite standard}) - \text{blank}}$$
 - Pour the 5 mL standards ($100 \mu\text{g/mL}$) through the cadmium column using the *Water Sample Processing Procedure* section below in the following order; blank, nitrate, nitrite, and nitrate.
 - Calculate the reduction efficiency of the column at $100 \mu\text{g/mL}$ as follows:
$$\text{RE} = \frac{(\text{averaged absorbencies of nitrate standards}) - \text{blank}}{(\text{absorbance of nitrite standard}) - \text{blank}}$$

Cleaning and Repacking the Cadmium Columns

- Clean and repack the cadmium columns with the following procedure:
 - Remove the cadmium granules by inverting the column into a 600 mL beaker and rinsing with DI water.
 - Add 50 mL of 10% HCl to the cadmium, swirl, and soak for ~ 30 minutes.
 - Rinse the cadmium with DI water, add ~30 mL of cupric sulfate solution, and soak for 15 minutes.

- d. Pour off the cupric sulfate solution, rinse the cadmium with DI water, and add 30 mL of cupric sulfate solution. Soak until the cadmium is coated with copper; i.e., when the blue color disappears. Rinse the cadmium thoroughly with DI water.
- e. After cleaning the columns thoroughly, close the outlet valve, and fill the column with DI water. Place a small wad of glass fiber wool into the reservoir and allow it to settle to the bottom of the column (Figure 6).
- f. Using a DI wash bottle, rinse the cadmium into the column so that the granules do not bypass the glass wool and the column is not obstructed with air bubbles.
- g. Re-check the RE.

Calculating Column Equations

1. Label two SC for each of the three remaining serial dilutions listed in Table 5 for each column. The other three serial dilutions were run as part of the RE.
2. Prepare the three remaining nitrate standards by adding the appropriate volume of secondary nitrate, dilute each to 50 mL with DI water, and add 5 mL of buffer. Invert the SC to mix.
3. Pour the serial dilutions through the cadmium column using the *Water Sample Processing Procedure* section below.
4. Formulate a linear equation for each column by regressing the nitrate serial dilution concentrations against their respective absorbances minus the average blank (0.000) serial dilution absorbance, and calculate the r^2 . Each column has a different regression formula and blank. Follow the procedures under the *Formulating Linear Equations* section. Refer to Figure 7 for an example plot of the serial dilutions listed in Table 5.

Water Sample Processing Procedure

1. Label two 50 mL SC for each water sample. When checking the RE or calculating column equations start at step 3.
2. Pour 50 mL of the filtered frozen water sample into a 50 mL SC and add 5 mL of buffer solution. Invert the SC to mix.
3. Pour ~ 20 mL of the buffered sample into the column reservoir, allow the sample to drip through, and discard the effluent. Make sure the duplicate samples from each lake are run through the same columns.
4. Pour the remaining sample (~35 mL) into the column reservoir and collect 30 mL of the effluent in the original cylinder. Allow the remaining sample (~5 mL) to drip through, and discard.
5. Add 0.5 mL of sulfanilamide solution to the 30 mL sample, and invert twice to mix. Allow 5 minutes for proper reaction time.
6. Add 0.5 mL of NNED solution and invert twice to mix. Allow 15 minutes for full color development. The color remains stable for hours.
7. Measure the serial dilution and/or sample absorbance against a DI water blank at 543 nm.

Note:

- For monthly maintenance or after extended periods without use, pass a concentrated buffer solution (25 mL buffer, 75 mL DI water) through the column.
- The volume of cadmium within a column greatly affects RE and flow rate. A volume of less than 18.5 cm reduces flow rates and leaves residual sample in the column contaminating the next sample and reducing RE. If residual nitrate is suspected, pass a 50-mL buffered blank (50 mL DI water with 5 mL buffer added) through the columns between sample sets to compensate for residual nitrogen left in the column.
- If the sample is tinted blue after passing through the column, clean the cadmium column.
- Keep the reservoir covered with parafilm at all times.

Calculations

1. Determine the water sample concentration by subtracting the average blank (0.000) serial dilution absorbance from the average water sample absorbance and insert the value into the regression formula provided by the serial dilutions. Each column has a different regression formula and blank value. For nitrate concentrations greater than $100 \mu\text{g L}^{-1}$ water samples must be diluted.

TOTAL PHOSPHOROUS (TP) AND TOTAL FILTERABLE PHOSPHOROUS (TFP)

Phosphorous concentrations in aquatic systems are typically low and can limit production (Koenings et al. 1987). N:P ratios $> 18:1$ are favorable for algae growth. Concentrations average $9 \mu\text{g L}^{-1}$ TP and $3 \mu\text{g L}^{-1}$ TFP for Kodiak Lakes.

Equipment

The following equipment is required: Genesys 5 Spectrophotometer, autoclave (121°C , 15 psi), 50 mL volumetric flasks, 50 mL SC, and 100 mm cuvettes. Quartz cuvettes are recommended over glass cuvettes.

Chemical Handling and Disposal

The antimony tartrate solution, digestion reagent, and concentrated acids are very hazardous to the health. All of these solutions require special handling and disposal. Consult MSDS for specifics.

Reagents

1. Antimony-tartrate solution – Add 53.3 mL of concentrated sulfuric (H_2SO_4) acid to ~800 mL of DI water and allow to cool at room temperature. Dissolve 0.748 g of antimony potassium tartrate trihydrate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$) in the sulfuric acid solution and dilute to 1 L with DI water. Filter the solution when a precipitate forms. Store refrigerated and replace every two months.
2. Molybdate solution – Dissolve 7.95 g of ammonium molybdate-4-hydrate ($(\text{NH}_4)\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$) in ~500 mL of DI water and dilute to 1 L with DI water. Store refrigerated and replace every two months.

3. 3.6 N Sulfuric acid – Add 12.5 mL of concentrated sulfuric acid (H_2SO_4) to ~ 100 mL of DI water, cool, and dilute to 125 mL with DI water.
4. Digestion reagent – Add 50 mL of 3.6 N sulfuric acid (H_2SO_4) to ~ 400 mL of DI water and dissolve 30 g of potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), dilute to 500 mL with DI water. Avoid sediment when pipeting.
5. Mixed reagent I (MRI) – Combine 125 mL of both the antimony-tartrate and molybdate solutions. Add 1 g of ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), dissolve, and dilute to 500 mL with DI water.

To decrease variability between sample runs the NIL only uses the digestion and mixed reagent I on the day after they were made.

Standards

1. Primary phosphorous standard ($0.05 \mu\text{g mL}^{-1} \text{P}$) – Dissolve 0.1389 g of potassium phosphate-dibasic (K_2HPO_4) in ~ 400 mL of DI water and dilute to 500 mL with DI water in a volumetric flask. The primary standard should be replaced after six months.
2. Secondary standard ($0.5 \mu\text{g mL}^{-1} \text{P}$) – Dilute 1 mL of the primary phosphorus standard to 100 mL with DI water in a volumetric flask. The secondary standard should be replaced after three months.

Procedure

1. Label two acid washed 50 mL volumetric flasks (VF) for each water sample, two acid washed VF for 0.00 and 1.00 serial dilutions (DI water blanks), and one acid washed VF for each of the 4 remaining serial dilutions listed in Table 6. Run serial dilutions each day samples are processed.
2. Use unfiltered water for TP and filtered water for TFP. Pour 25 mL of the sample water, or 25 mL of serial dilution into a 50-mL VF. Process water samples and serial dilutions simultaneously.
3. Add 5 mL of digestion reagent and mix. Cover the VF with aluminum foil and let it digest in the autoclave for 30 minutes. Follow the autoclave procedure below.
 - a. Leave the temperature setting on low and set the autoclave timer to 50 minutes to allow for the slow cooling cycle.
 - b. Fill the water reservoir with distilled water.
 - c. Turn on the power and the slow exhaust switches.
 - d. When the timer sounds, slowly crack open the autoclave door to allow venting.
4. Cool the samples to room temperature. Add 5 mL of MRI and mix. Add the reagent to the volumetric flasks in groups of three every ten minutes to stagger color development, allowing for proper processing time.
5. Allow exactly 20 minutes for full color development. Readings are time dependent. Measure the serial dilution and water sample absorbance against a DI water blank at 882 nm.

Calculations

1. Formulate a linear equation by regressing the phosphorous serial dilution concentrations against their respective absorbances minus the average blank (0.000) serial dilution absorbance, and calculate the r^2 . Follow the procedures under the *Formulating Linear Equations* section. Refer to Figure 8 for an example plot of the serial dilutions in Table 6.
2. Determine the water sample concentration by subtracting the average blank (0.000) serial dilution absorbance from the average water sample absorbance and insert the value into the regression formula provided by the serial dilutions.

FILTERABLE REACTIVE PHOSPHOROUS (FRP)

Algae readily take up filterable reactive phosphorous, commonly called soluble inorganic orthophosphate (Koenings et al. 1987).

Equipment

The following equipment is required: Spectronic Genesys Spectrophotometer, 50-mL stoppered cylinders, 100-mm cuvettes (quartz recommended).

Chemical Handling and Disposal

The antimony tartrate solution and concentrated acids are very hazardous to the health. All of these solutions require special handling and disposal. Consult MSDS for specifics.

Reagents

1. Prepare reagents 1 through 3 as described in the TP and TFP methods.
2. Mixed reagent II (MRII) – Combine in order: 125 mL of molybdate solution, 50 mL of 3.6 N sulfuric acid (H_2SO_4), and 125 mL of antimony-tartrate solution. Add 1.0 g of ascorbic acid ($C_6H_8O_6$), allow the solution to dissolve, and dilute to 500 mL with DI water.

To decrease variability between sample runs the NIL only uses the MR II on the day after it was made.

Standards

Prepare the primary and secondary standards as described in the TP and TFP methods.

Procedure

1. Label two acid washed 50-mL SC for each water sample, two acid washed SC for 0.0 (DI water blanks) and 0.50 serial dilutions, and one acid washed SC for each of the four remaining serial dilutions listed in Table 7. Run serial dilutions each day samples are processed.
2. Pour 25 mL of filtered frozen water sample or 25 mL of serial dilution into an acid washed 50 mL SC. Process sample water and serial dilutions simultaneously.
3. Add 5 mL of MRII, and mix. Add the reagent to the SC in groups of three every ten minutes to stagger color development, allowing for proper processing time.
4. Allow exactly 20 minutes for full color development. Readings are time dependent. Measure the serial dilution and water sample absorbance against a DI water blank at 882 nm.

Calculations

1. Formulate a linear equation by regressing the phosphorous serial dilution concentrations against their respective absorbances minus the average blank (0.000) serial dilution absorbance, and calculate the r^2 . Follow the procedures under the *Formulating Linear Equations* section. Refer to Figure 9 for an example plot of the serial dilutions listed in Table 7.
2. Determine the water sample concentration by subtracting the average blank (0.000) serial dilution absorbance from the average water sample absorbance and insert the value into the regression formula provided by the serial dilutions.

CHLOROPHYLL *a* AND PHAEOPHYTIN *a*

Primary production can be estimated by calculating the concentration of phytoplankton chlorophyll pigments in a volume of water (Edmonson 1959). Chlorophyll *a* is the largest component of chlorophyll algal pigments. Degradation of chlorophyll results in increased presence of phaeophytin *a* (Lind 1985).

Equipment

The following equipment is required: Genesis 5 Spectrophotometer, grinding vessel (ceramic mortar and pestle), Clay-Adams Dynac centrifuge, 15-mL glass centrifuge tubes, Whatman GF/F filter pads, and 10 mm cuvettes (quartz recommended).

Chemical Handling and Disposal

Sodium bicarbonate is mildly hazardous, requiring no special handling or disposal. Acetone use should be confined to the hood. Wash hands after use and avoid extended contact. Hydrochloric acid is very reactive and a health hazard, prevent contact and handle or dispose of only in the fume hood. Consult MSDS for specifics.

Reagents

1. 1 N Sodium bicarbonate – Dissolve 8.4 g of sodium bicarbonate (NaHCO_3) into 100 mL DI water.
2. 2 N Hydrochloric acid – Add 42 mL of concentrated hydrochloric acid (HCl) to ~ 200 mL of DI water and dilute to 250 mL with DI water.
3. 90% neutralized acetone – Add 1 mL of 1 N sodium bicarbonate to 900 mL of reagent-grade acetone ($\text{C}_3\text{H}_6\text{O}$) and dilute to 1 L with DI water and filter in the hood.

Procedure

1. Partially thaw filter pads, place into a chilled mortar, and add 2 mL of 90% acetone.
Note: Keep the samples dark and as cold as possible throughout the procedure. Storing the mortar in the freezer helps keep the samples cold. Avoid washing glassware with 10% HCl, use the 90% neutralized acetone.
2. Carefully grind the filter pad into a slurry. Add 2 mL 90% acetone and regrind.
3. Scrape the slurry into a 15 mL centrifuge tube, rinse the mortar and pestle with ~ 4 mL of 90% acetone and add the contents to the centrifuge tube. Rinse the mortar and pestle again

with 2 mL of 90% acetone, and add the contents to the centrifuge tube. The total volume of acetone added should be 10 mL. All centrifuge tubes should be filled to the same level to balance the centrifuge.

4. Cover the centrifuge tube with a cap, and refrigerate the samples for 2-3 hours to complete chlorophyll *a*-extraction. Samples can be stored in the refrigerator overnight to allow for increased production. Keep the centrifuge tubes covered and dark.
5. Centrifuge the tubes for 40 minutes at 2,500 rpm. Decant the supernatant into a 25 mL graduated cylinder and dilute to 12 mL with 90% acetone.
6. Invert to mix, split equally (6 mL) into two centrifuge tubes, add 0.05 mL of 2 N HCl to one tube. Invert to mix the acidified centrifuge tube.
7. Measure the absorbance of the unacidified fraction against a 90% acetone blank at 750 nm, 665 nm, 663 nm, 645 nm, and 630 nm.
8. Measure the absorbance of the acidified fraction against a 90% acetone blank at 750 nm, and 665 nm.

Calculations

(Monochromatic method)

$$\text{chlorophyll } a \text{ (}\mu\text{g L}^{-1}\text{)} = \frac{26.7 (665_o - 665_a) \times V_s}{L \times V_f}$$

$$\text{phaeophytin } a \text{ (}\mu\text{g L}^{-1}\text{)} = \frac{26.7 (1.7 (665_a) - 665_o) \times V_s}{L \times V_f}$$

(665_o) = absorbance 665 nm – absorbance 750 nm (before acidification).

(665_a) = absorbance 665 nm – absorbance 750 nm (after acidification).

V_s = total volume (mL) of sample extract (12 mL normally).

L = path length (cm) of cuvette.

V_f = volume (L) of lake water filtered

ZOOPLANKTON

The zooplankton community is the primary forage base for juvenile sockeye salmon and therefore its characteristics are important in gauging rearing conditions and evaluating results of stocking or enrichment programs (Koenings et al. 1987). Salmon fry exert predation pressure that can structure the zooplankton community; although environmental conditions also play a role in community structure (Lind 1985).

Equipment

The following equipment is required: microscope with ocular micrometer, Sedgwick-Rafter counting cell (S-R Cell), 1 mL or Henson-Stemple pipet, small funnel, a section of 153 micron mesh plankton net cut from an old sampling net large enough to fit in the funnel, magnetic stirrer, and 250 mL beaker.

Chemical Handling and Disposal

Formalin is a health hazard. Always avoid contact or fumes and dispose of waste in the hood. Consult MSDS for specifics.

Procedure

1. Place the section of cut plankton net onto the funnel over a formalin waste container and empty the contents of the plankton sample bottle onto the net. Rinse the plankton sample bottle several times with DI or tap water and pour onto the net to remove all plankton from the bottle.
2. Invert the net over a 250 mL beaker and rinse the contents into the beaker using a known volume of DI water.
3. Dilute the contents of the beaker to ~100 to 150 organisms per mL. Do a quick count in a S-R Cell if uncertain about the organism density in the beaker. Be sure to empty the S-R Cell contents back into the beaker and rinse all the zooplankters off the S-R Cell. Add more water if needed, recording the final volume.
4. Slowly mix the sample with a magnetic stirrer and draw out a random 1 mL sample. Mix gently and only as long as needed to get an even mix because over-mixing will detach eggs or egg clusters from ovigerous zooplankters. Place the sample onto a clean S-R Cell with a cover slip.
5. Using the microscope, identify, measure, and count the zooplankters present on the S-R Cell. Count at least 3 of the 1 mL aliquots. Dispose of the counted aliquots. Returning the counted aliquots to the beaker would require rinsing of the S-R Cell to get all the zooplankters off the S-R Cell. Rinsing the S-R Cell into the beaker changes the dilution.

Note: Lines on the S-R counting cells are used to facilitate systematic counting. Counts from the 3 aliquots should be similar. Additional counts may be necessary to achieve consistency if the aliquots contain less than 100 to 150 zooplankters or if counts vary greatly. To store a sample, replace the water with 10% neutralized formalin.

Identification

Common cladocerans in Alaska lakes include *Bosmina*, *Daphnia*, *Holopedium*, *Chydorinae*, and *Polyphemus* (Table 8). Common copepods (Copepoda) include *Cyclops*, *Diaptomus*, *Epischura*, and *Harpacticus* (Table 8) and some common rotifers include *Kellicottia*, *Asplanchna*, *Keratella*, *Conochilus*, and *Filinia*.

To follow historical data collection classifications, the NIL keys common macrozooplankton to the following taxonomic levels (most organisms identified to genus are comprised entirely of one species):

- Species: The cladocerans *Holopedium* and *Daphnia* are keyed to species.
- Genus: The cladocerans *Bosmina*, *Chydorinae*, and *Polyphemus* are keyed to genus. The copepods *Epischura*, *Cyclops*, *Diaptomus*, and *Harpacticus* are keyed to genus. The rotifers *Kellicottia*, *Keratella*, *Asplanchna*, *Conochilus*, and *Filinia* are keyed to genus.

Edmonson (1959) is used as reference for zooplankton identification. Drawings of the most common cladocerans and copepods are shown in Figure 10. These two groups of zooplankton

are the key genera identified. Occasionally, organisms such as rotifers, ostracods, oligochaetes, and insects are also counted. Additional useful references for invertebrate identification include Sherman and Sherman (1976), Einsle (1996), and Smith (1977). For insect identification use McCafferty (1983).

Differentiation between cladocerans and copepods is fairly easy. Cladocerans are unsegmented and round in shape, with branching antennae. Copepods are segmented and elongate without branching antennae.

Identify cladocerans to species when possible. The gelatinous mass around *Holopedium* indicated in Figure 10, is rarely visible. Ovigerous (ovig) cladocerans are counted and measured separately. Ovigerous cladocerans store eggs in a brood pouch within the body which are visible as darker structures. There may be cladocerans that have hatched from the brood pouch, but are not identifiable yet. These immature cladocerans are counted, but not measured. Make certain all zooplankton readers are consistent with this identification.

Differentiation among copepods can be difficult. Egg bearing (ovigerous) copepods are counted and measured separately. Ovigerous cyclopoids have one egg sack on each side of their body, towards the tail end. Ovigerous calanoids have one egg sack centrally located towards the tail end. Early life stage copepods are called nauplii. Nauplii have not matured enough to allow for differentiation between species groups (Figure 11). Nauplii are counted, but not measured.

Miscellaneous species are counted but not measured. If new species are encountered, count them and inform the project biologist.

Measurements

1. The microscopes the NIL uses have been calibrated. If a new microscope is used, calibrate the microscope with an ocular micrometer to determine if any conversions are necessary. For calibration the ocular micrometer is placed in the microscope instead of the slide and the lines on the ocular micrometer are matched with the microscope eye piece grids or lines.
2. Measure the distance from the top of the head to the end of the carapace (Figure 10) for the first 15 individuals of each copepod or cladoceran species encountered. Rotate the microscope eye piece grid for proper orientation. If an individual zooplankter is not suitable for measurement (i.e. broken, distorted, or oriented to give an inaccurate measurement) measure the next individual encountered. Remember to separately measure ovigerous individuals. Immature cladocerans and nauplii do not need to be measured. Record lengths to the nearest 0.01 mm if possible.
3. Determine the required number of organisms to measure resulting in a level of precision of $\pm 10\%$ at a 95% confidence level using a student's t-test. Excel spreadsheet Template 1, determination of sample number for adequate body size measurement, (Appendix A4) and Table 9 are used to give the number of measurements needed. Changes to formulas in the templates require a password.

Zooplankton sample size determination for body size (Template 1)

1. Enter organism, lake, and date.
2. Enter the first 15 measurements taken.
3. Mean and standard deviation (S.D.) are automatically computed.

4. Using n , $n-1$ is automatically computed.
 5. Using $n-1$, find the corresponding t -statistic from Table 9 and enter it in the correct cell.
 6. This will give you N , which is the number of organisms that should be measured from that sample if possible.
4. Measure additional zooplankters if needed to reach N , given by the student's t -test. If insufficient measurements are attained after processing the three aliquots, do a fourth slide. Do not stir the sample for the fourth slide so you get a concentration of zooplankters. Scan the slide for the needed measurements.

Template 1 calculates:

- g. The mean length (L) and standard deviation (SD) of the first 15 zooplankters of a species, and determines n by substituting into the formula:

$$n = [(1.96 \times SD) / (0.10 \times L)]^2$$

- h. The number (N) of zooplankters to be measured by using $n-1$ and the t -statistic at a confidence level of 95% (Table 9) and substituting t , SD , and L into the formula:

$$N = [(t \times SD) / (0.10 \times L)]^2$$

N = number to be measured

4. Record the length measurements on the Zooplankton Data Sheet (Appendix A5).

Counting

1. Systematically count how many organisms of each type or category are present on the S-R Cell. The entire S-R Cell should be counted for accurate and consistent results. Enter the counts on the Zooplankton Data Sheet (Appendix A5). Count a minimum of three sample aliquots.
2. Excel spreadsheet Template 2 (Appendix A6) is used for count and measurement calculations.

Changes to formulas in the templates require a password. Contact authorized personnel if new organisms or errors are found. Follow the proceeding instructions to complete Template 2 (a,b, and c). Templates 3 (Appendix A7) and 4 (Appendix A8) are automatically completed.

- a. Enter sample data (lake, station, depth, and date) into the appropriate cells.
- b. Enter the sample dilution volume into the appropriate cell.
- c. Enter the count and length results from each aliquot into the appropriate species row. Enter a zero if a species/genus was not found in the aliquot. Be sure to use the appropriate size (e.g. a *Bosmina* of 0.31 mm). If you have no length measurement, leave the cell blank.

The remainder is automatically calculated.

- d. Total in sample column: This is the mean multiplied by the dilution factor.

- e. #/m²: This is the total in sample divided by the area of the plankton net opening. Make sure the area is correct for the plankton net used for that tow (0.2 m net = 0.0314; 0.5 m net = 0.1963).

$$\text{Zooplankter per m}^2 = \frac{\text{\# in entire sample}}{\text{Net area (m}^2\text{)}}$$

- f. #/m³: This is the total in sample divided by the product of the depth of tow and the area of the plankton net opening. Again, check the net area formula.

$$\text{Zooplankter per m}^3 = \frac{\text{\# in entire sample}}{\text{Depth (m) of tow x net area (m}^2\text{)}}$$

Body size and biomass sections (Template 3, Appendix A7)

- Weighted mean length: The formula uses the mean body size of an organism combined with how numerous that organism was on the given sample date, and gives an adjusted mean length.
- Biomass: This uses the total mean length and a wet length to dry weight relationship specific to each organism (Table 8) to determine biomass. Make sure you are using the correct equation for the organism.
- Weighted biomass: Identical to biomass, except using the weighted mean length.
- Group weighted biomass and group weighted length are simply the sums of ovigerous and non-ovigerous organisms.

QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

To ensure precise and accurate data, quality assurance (QA) and quality control (QC) measures must be taken.

INTERNAL QA AND QC MEASURES

- General laboratory procedures will be followed to ensure the cleanliness of all glassware, the exact preparation and addition of reagents or standards, and the use of serial dilutions with every nutrient batch.
- Track reagent changes by plotting reagent age (days) against a standardized absorbance value (for example, Table 2, Figure 3). A linear equation with a concentration value outside of an R² of 0.90 for the corresponding reagent age is unacceptable and will require the test to be redone (for example, Figure 3). Large Y intercepts are also unacceptable requiring the test to be redone. As reagents age, a given absorbance will give different values. Most of our tests show a decreasing exponential relationship. Absorbances for TP/TFP show an increasing relationship.
 - Choose a standardized absorbance value and enter the value into column A. The standardized absorbance value chosen should reflect an average corrected absorbance value from the data.
 - Enter the actual linear formulas for the specific test into column B.

- c. Enter the corresponding age (days) of the reagent for the linear formula into column C.
- d. Enter the value calculated by substituting the standardized absorbance value into the linear equation in column D.
- e. Plot the reagent days against the calculated value using a scatter plot (follow the procedures under the *Formulating Linear Equations* section) to get a linear equation and r^2 .

EXTERNAL QA AND QC MEASURES

1. Use certificate of analysis nutrient samples: these nutrients come in a prepared ampule with the reference value in a sealed envelope. The certificate of analysis provides an advisory range from the National Standards for Water Proficiency Testing Studies Criteria Document (1998) at the 95% confidence level.
2. Send duplicate water samples to ADF&G's CRL program or South Dakota University for analysis. CRL participates in the U.S. Geological Survey's analytical evaluation program for standard reference samples.

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REFERENCES CITED

- ADF&G. 2002. Salmon research operational plans for the Kodiak area, 2002. Kodiak Island lake assessment/limnology project laboratory analyses operational plan. Alaska Department of Fish and Game, Commercial Fisheries Division, Regional Information Report 4K20-36, Kodiak.
- ADF&G. 2007. Salmon research operational plans for the Kodiak area, 2007. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 4K07-7, Kodiak.
- Corning instruction manual. 1996. Corning Incorporated Science Products Division. New York.
- Edmondson, W.T. (ed.) 1959. *Freshwater biology, second edition*. John Wiley and Sons, Inc. USA.
- Einsle, U. 1996. Guides to the identification of the microinvertebrates of the continental waters of the world volume 1: Copepoda: Cyclopoida: genera Cyclops, Magacyclops, Acanthocyclops. SPB Academic Pub., Amsterdam, Netherlands.
- Honnold, S.G. and J.A. Edmondson. 1996. Limnological and fishery assessment of 23 Alaska Peninsula and Aleutian area lakes, 1993-1995: an evaluation of potential sockeye and coho salmon production. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 4K96-52, Kodiak.
- Honnold, S.G., and S. Schrof. 2004. Stock assessment and restoration of the Afognak Lake sockeye salmon run. Fisheries Resource Monitoring Program. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fishery Information, Services Division, Final Project Report No. FIS 03-047, Anchorage.
- KRPT. 1992. Kodiak regional comprehensive salmon plan, 1982-2002: phase II revision. Alaska Department of Fish and Game, Office of the Commissioner, Juneau.

REFERENCES CITED (Continued)

- Koenings, J.P., J.A. Edmundson, G.B. Kyle, and J.M. Edmundson. 1987. Limnology field and laboratory manual: methods for assessing aquatic production. Alaska Department of Fish and Game, FRED Division Report Series 71. Juneau.
- Lind, O.T. 1985. Handbook of common methods in limnology. Kendall/Hunt publishing company. USA.
- Metler Toledo instruction manual. 2003. Metler-Toledo GmbH, Analytical, Sonnenbergstrasse 74, Printed in China.
- McCafferty, W.P. 1983. Aquatic entomology; the fishermen's and ecologists illustrated guide to insects and their relatives. Jones and Bartlett publishers, Inc. USA.
- National standards for water proficiency testing studies criteria document. December 1998. EPA. USA.
- Schrof, S.T., S.G. Honnold, C.J. Hicks and J.A. Wadle. 2000. A summary of salmon enhancement, rehabilitation, evaluation, and monitoring efforts conducted in the Kodiak management area through 1998. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report 4K00-57, Kodiak.
- Sherman, I.W. and V.G. Sherman. 1976. The invertebrates: function and form a laboratory guide, *second edition*. Macmillan Publishing Co., Inc. USA.
- Smith, D.L. 1977. A guide to marine coastal plankton and marine invertebrate larvae. Kendall/Hunt publishing company. USA.
- Spectronic Genesys spectrophotometer manual. 1996. Spectronic Instruments, Inc. Rochester, New York.
- Standard methods for the examination of water and wastewater, *20th Edition*. 1998. American Water Works Association, Washington D.C.
- Wetzel, R.G. and G.E. Likens. 1991. Limnological analyses, *second edition*. Springer-Verlag New York Inc. New York.

TABLES AND FIGURES

Table 1.–Formulating a linear equation using total ammonia (TA) serial dilutions.

Volume of secondary nitrogen standard (mL) to be added	Volume of DI water (mL) to be added	Ammonia concentration (ug/mL)	Sample absorbance (x) at 640 nm	Corrected absorbance (x-blank)
0.0	50.0	0.0	0.036	0.000
0.1	49.9	1.0	0.053	0.017
0.3	49.7	3.0	0.058	0.022
0.5	49.5	5.0	0.066	0.030
1.5	48.5	15.0	0.127	0.091
3.0	47.0	30.0	0.225	0.190
5.0	45.0	50.0	0.299	0.263

Note: Sample absorbance of 0.0 Ammonia concentration is the "blank"

Table 2.–Total ammonia reagent age versus. nutrient concentration values.

Standardized absorbance value (SAV)	Calculated linear formulas	Reagent age in days (phenol)	Concentration calculated from entering SAV into linear formula (TA)
X = .100	y=154.47x-0.2372	1	15.2
X = .100	y=151.57x-4.5252	2	10.6
X = .100	y=136.31x-9.6577	5	4.0

Table 3.–Serial dilutions needed for color testing.

Volume of color standard (mL) to be added	Volume of DI water (mL) to be added	Platinum Cobalt Unit (PT) concentration	Sample absorbance at 400 nm
0.00	4.00	0.0	0.000
0.10	3.90	12.5	0.019
0.20	3.80	25.0	0.046
0.30	3.70	37.5	0.064
0.50	3.50	62.5	0.103
1.00	3.00	125.0	0.210
2.00	2.00	250.0	0.420
3.00	1.00	375.0	0.615
3.50	0.50	437.0	0.726
3.70	0.30	462.0	0.769
3.80	0.20	475.0	0.786
3.90	0.10	487.0	0.803
4.00	0.00	500.0	0.826

Table 4.–Serial dilutions needed for total ammonia (TA) testing.

Volume of secondary nitrogen standard (mL) to be added	Volume of DI water (mL) to be added	Ammonia concentration (ug/mL)	Sample absorbance (x) at 640 nm	Corrected absorbance (x-blank)
0.0	50.0	0	0.036	0.000
0.1	49.9	1	0.053	0.017
0.3	49.7	3	0.058	0.022
0.5	49.5	5	0.066	0.030
1.5	48.5	15	0.127	0.091
3.0	47.0	30	0.225	0.190
5.0	45.0	50	0.299	0.263

Note: Sample absorbance of 0.0 Ammonia concentration is the "blank"

Table 5.–Serial dilutions needed for Nitrate + Nitrite testing.

Volume of secondary nitrate standard (mL) to be added	Volume of DI water (mL) to be added	Nitrogen concentration (ug/mL)	Sample absorbance (x) at 543 nm	Corrected absorbance (x-blank)
0.0	50.0	0	0.025	0.000
0.1	49.9	2	0.073	0.048
1.0	49.0	20	0.579	0.554
2.5	47.5	50	1.109	1.084
5.0	45.0	100	2.400	2.375
10.0	40.0	200	3.290	3.265

Note: Sample absorbance of 0.0 Nitrate concentration is the "blank"

Table 6.–Serial dilutions needed for total phosphorous (TP) and total filterable phosphorous (TFP) testing.

Volume of secondary phosphorous standard (mL) to be added	Volume of DI water (mL) to be added	Phosphorous concentration (ug/mL)	Sample absorbance (x) at 882 nm	Corrected absorbance (x-blank)
0.00	25.0	0	0.032	0.000
0.10	24.9	2	0.046	0.014
0.20	24.8	4	0.056	0.024
0.30	24.7	6	0.086	0.054
0.50	24.5	10	0.086	0.054
1.00	24.0	20	0.128	0.096

Note: Sample absorbance of 0.0 Phosphorus concentration is the "blank"

Table 7.–Serial dilutions needed for filterable reactive phosphorous (FRP) testing.

Volume of secondary phosphorous standard (mL) to be added	Volume of DI water (mL) to be added	Phosphorous concentration (ug/L)	Sample absorbance (x) at 882 nm	Corrected absorbance (x-blank)
0.00	25.0	0	0.0065	0.0000
0.05	25.0	1	0.0130	0.0065
0.10	24.9	2	0.0180	0.0115
0.20	24.8	4	0.0290	0.0225
0.30	24.7	6	0.0470	0.0405
0.50	24.5	10	0.0710	0.0645

Note: Sample absorbance of 0.0 Phosphorus concentration is the "blank"

Table 8.–Wet length to dry weight relationships for calculating zooplankton biomass.

Taxa	Excel spreadsheet formulas for biomass using wet length to dry weight relationship a
Copepods	
<i>Ergasilis</i>	= (mean length ^{2.14} *0.0036*mean density)
<i>Epischura</i>	= (mean length ^{2.84} *0.0045*mean density)
<i>Diaptomus</i>	= (mean length ^{2.82} *0.0043*mean density)
<i>Heterocope</i>	= (mean length ^{2.88} *0.0055*mean density)
<i>Eurytemora</i>	= (mean length ^{1.61} *0.0058* mean density)
<i>Cyclops</i>	= (mean length ^{2.14} *0.0036*mean density)
<i>Harpacticus</i>	= (mean length ^{2.14} *0.0036*mean density)
Cladocerans	
<i>Bosmina</i>	= (mean length ^{2.11} *0.0102*mean density)
<i>Daphnia l.</i>	= (mean length ^{2.17} *0.0046*mean density)
<i>Daphnia g.</i>	= (mean length ^{3.31} *0.0025*mean density)
<i>Daphnia r.</i>	= (mean length ^{2.35} *0.0036*mean density)
<i>Holopedium g.</i>	= (mean length ^{2.44} *0.0114*mean density)
<i>Chydorinae</i>	= (mean length ^{2.11} *0.0102*mean density)
<i>Polyphemus</i>	= (mean length ^{1.49} *0.0009*mean density)

^a Data supplied by Jim Edmundson, personal communication.

Where, ^ (claret) in excel spreadsheets is used for raising a number to a power.

Table 9.—Student’s t-statistic and sample sizes (n) used to determine the number (N) of zooplankter’s to be measured to achieve a confidence level (CL) of 95%.

n-1	t-statistic (95% CL)	n-1	t-statistic (95% CL)	n-1	t-statistic (95% CL)
1	12.70	11	2.20	21	2.08
2	4.30	12	2.18	22	2.07
3	3.18	13	2.16	23	2.07
4	2.78	14	2.14	24	2.06
5	2.57	15	2.13	25	2.06
6	2.45	16	2.12	26	2.06
7	2.36	17	2.11	27	2.05
8	2.31	18	2.10	28	2.05
9	2.26	19	2.09	29	2.05
10	2.23	20	2.09	>or= 30	1.96

Taken from Koenings et al. (1987).

Detailed Lake Survey Field Form - Division of Commercial Fisheries, Limnology Section

Lake _____ Station _____ Bottom depth _____ (m)

Weather and lake surface conditions _____

Date _____ Name (s) of sampling personnel _____ Time _____

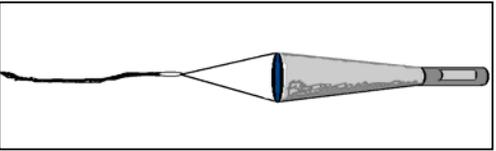
Physical Parameters				
Depth (m)	Temperature (°C)	Dissolved Oxygen (mg/l)	Depth (m)	Solar Illuminance (Foot Candles) UP
Incidence			Incidence	
Surface			Surface	
0.5			0.5	
1.0			1.0	
1.5			1.5	
2.0			2.0	
2.5			2.5	
3.0			3.0	
3.5			3.5	
4.0			4.0	
4.5			4.5	
5.0			5.0	
6.0			6.0	
7.0			7.0	
8.0			8.0	
9.0			9.0	
10.0			10.0	
11.0			11.0	
12.0			12.0	
13.0			13.0	
14.0			14.0	
15.0			15.0	
16.0			16.0	
17.0			17.0	
18.0			18.0	
19.0			19.0	
20.0			20.0	
21.0			21.0	
22.0			22.0	
23.0			23.0	
24.0			24.0	
25.0			25.0	
30.0			26.0	
35.0			27.0	
40.0			28.0	
45.0			29.0	
50.0			30.0	

Water samples:
 were / were not collected for lab analysis.
 Depths of samples: (m), (m) and (m)
Samples were filtered in the lab.

Zooplankton:
 One Vertical tow was taken at each station.
 Station No. _____ Bottom depth _____ (m)
 Zooplankton tow depth _____ (m)
 Station No. _____ Bottom depth _____ (m)
 Zooplankton tow depth _____ (m)
 Net size was **20cm x 153u**.
 Each tow was preserved separately in 10% Buffered Formalin.

Secchi disk:
 disappeared: _____ (m)
 reappeared: _____ (m)
 Mean disk reading: _____ (m)

Comments:



Hand held temp. _____ water: _____ air: _____
 DO Meter _____ Light Meter _____

Figure 1.--Detailed lake survey form.

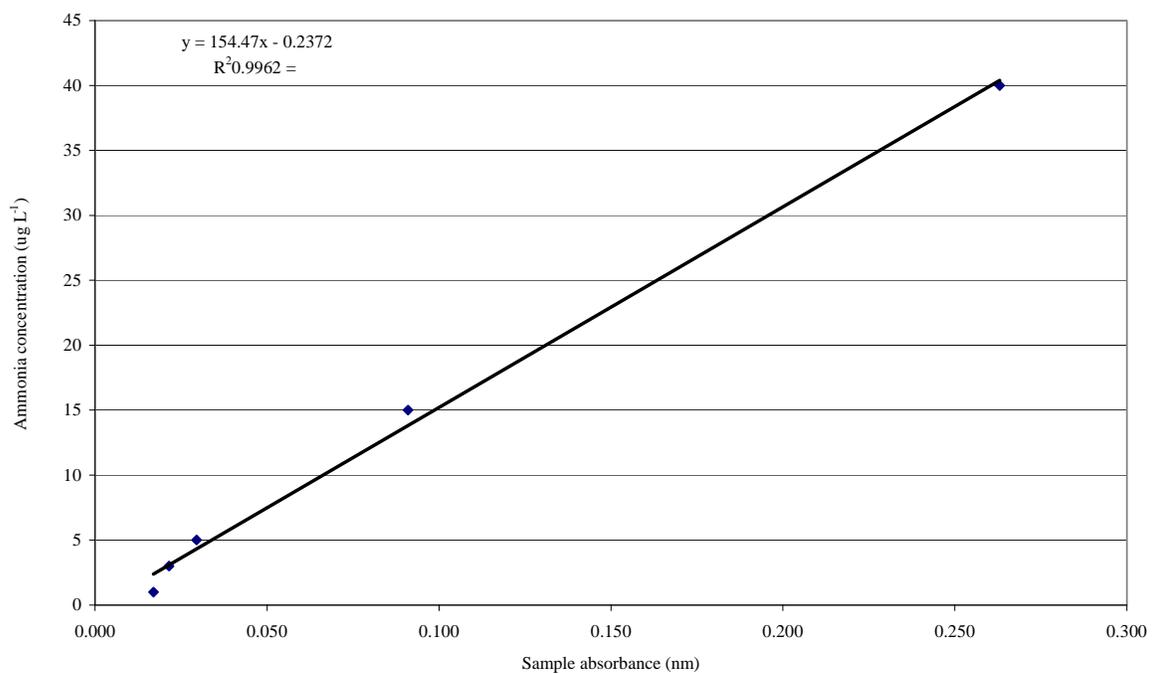


Figure 2.—A linear equation for total ammonia (TA) calculations.

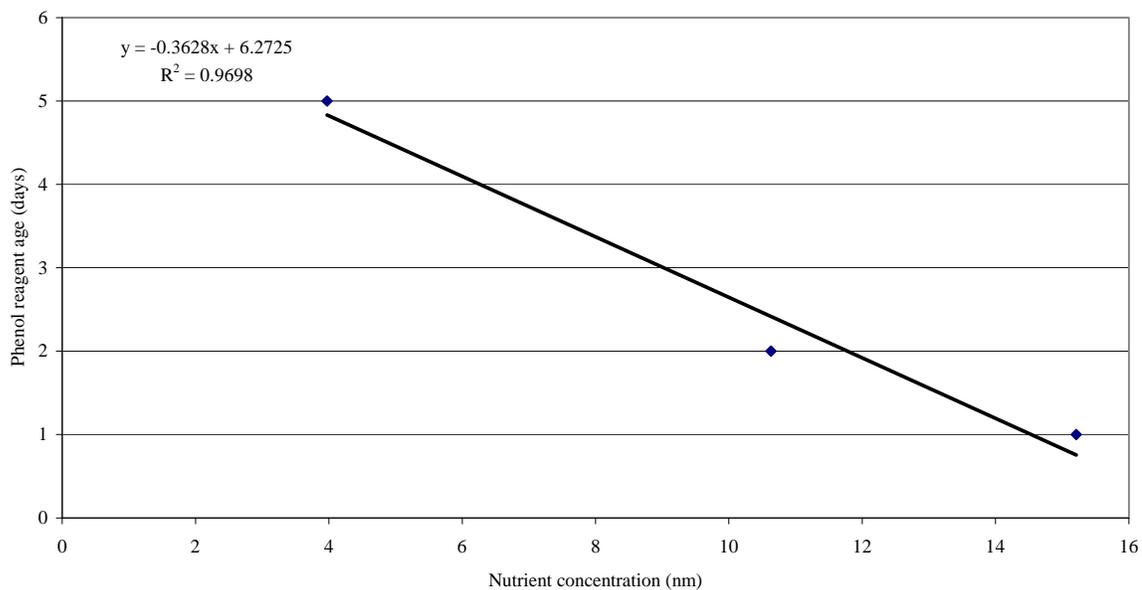


Figure 3.—Total ammonia reagent age versus nutrient concentration values.

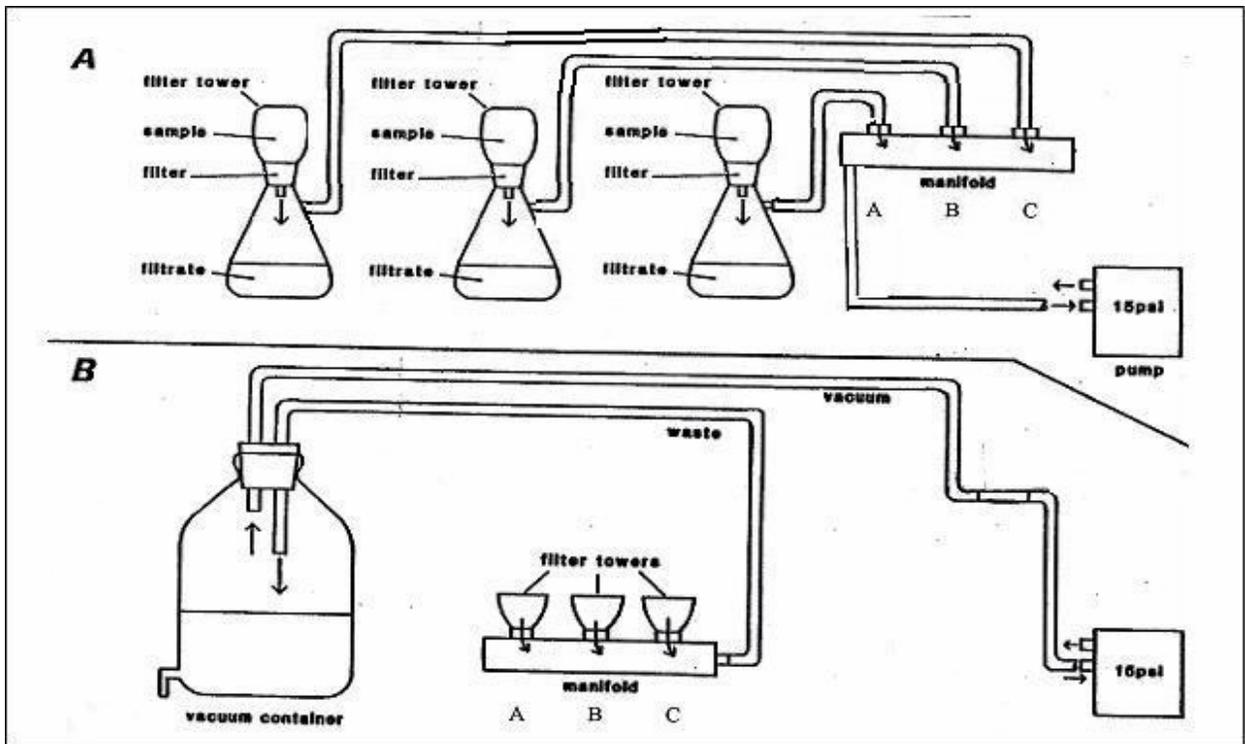


Figure adapted from Koenings et al. (1987).

Figure 4.—Apparatus used to process samples by vacuum filtration to obtain particulates and filtrate: A) using **three** filtrate flasks and B) using a filtering manifold.

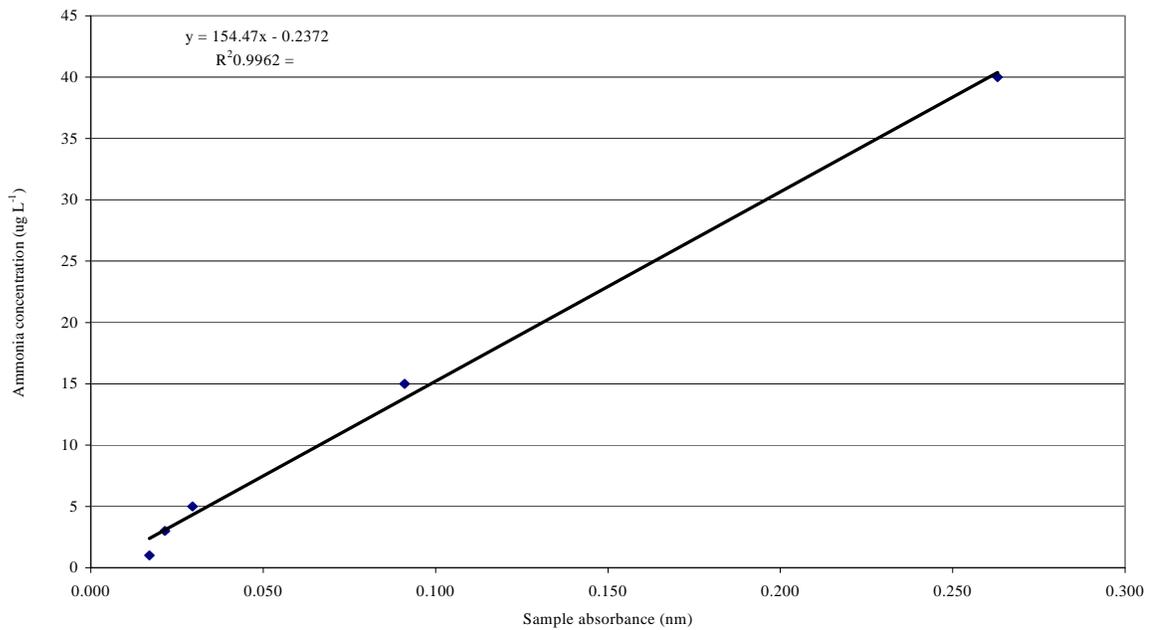
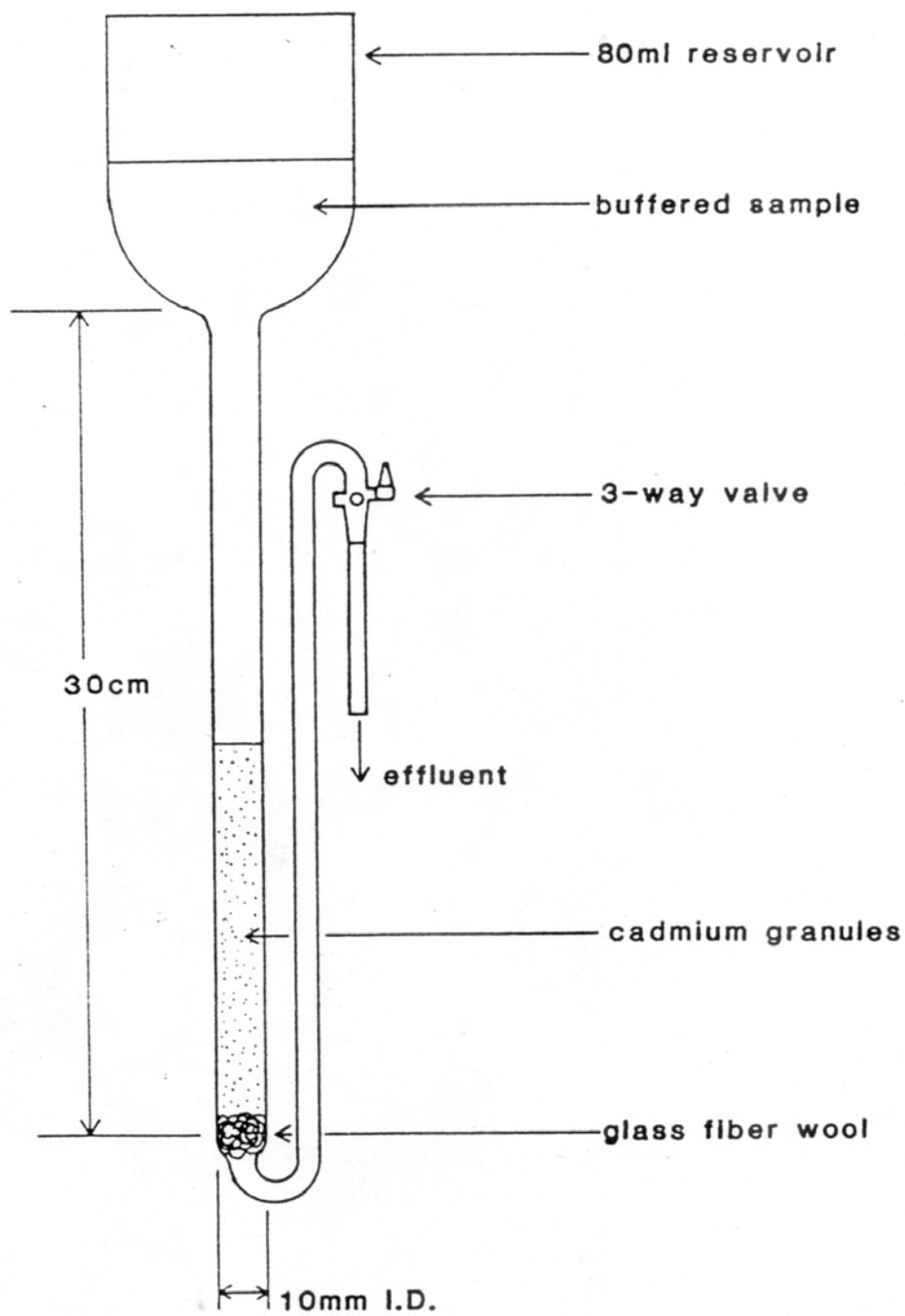


Figure 5.—Total ammonia (TA) concentration **versus** sample absorbance.



(Koenings et al. 1987).

Figure 6.—Cadmium column used to reduce nitrate to nitrite in the manual analysis of nitrate + nitrite nitrogen.

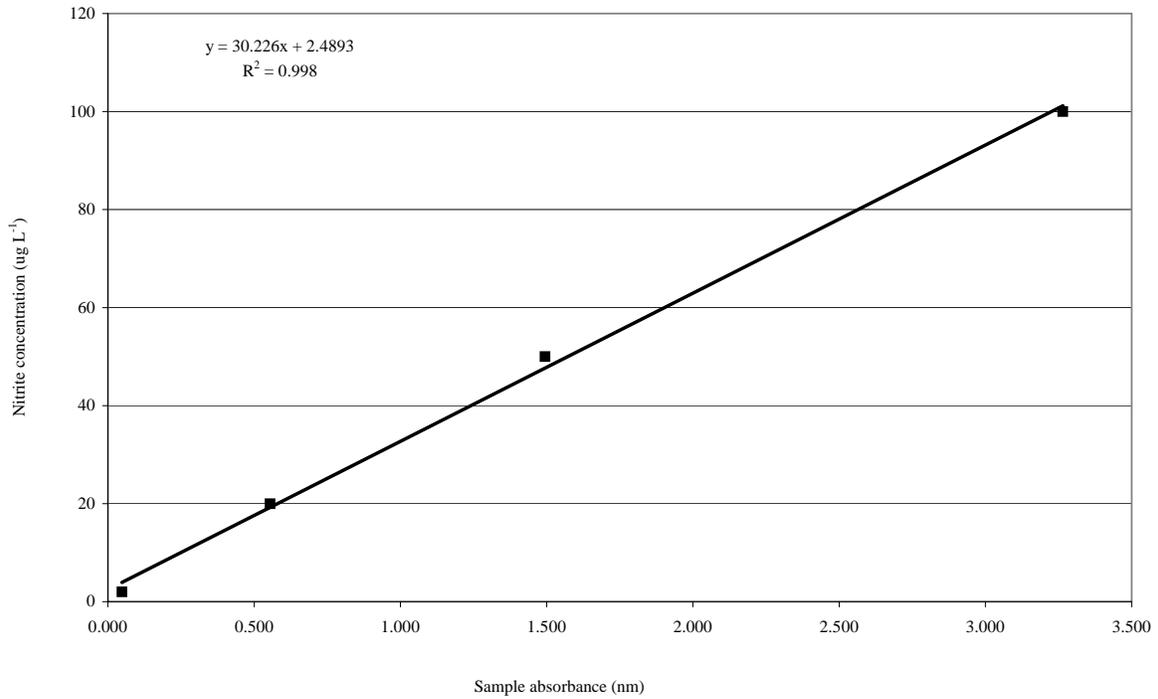


Figure 7.—Nitrate + nitrite concentration versus sample absorbance.

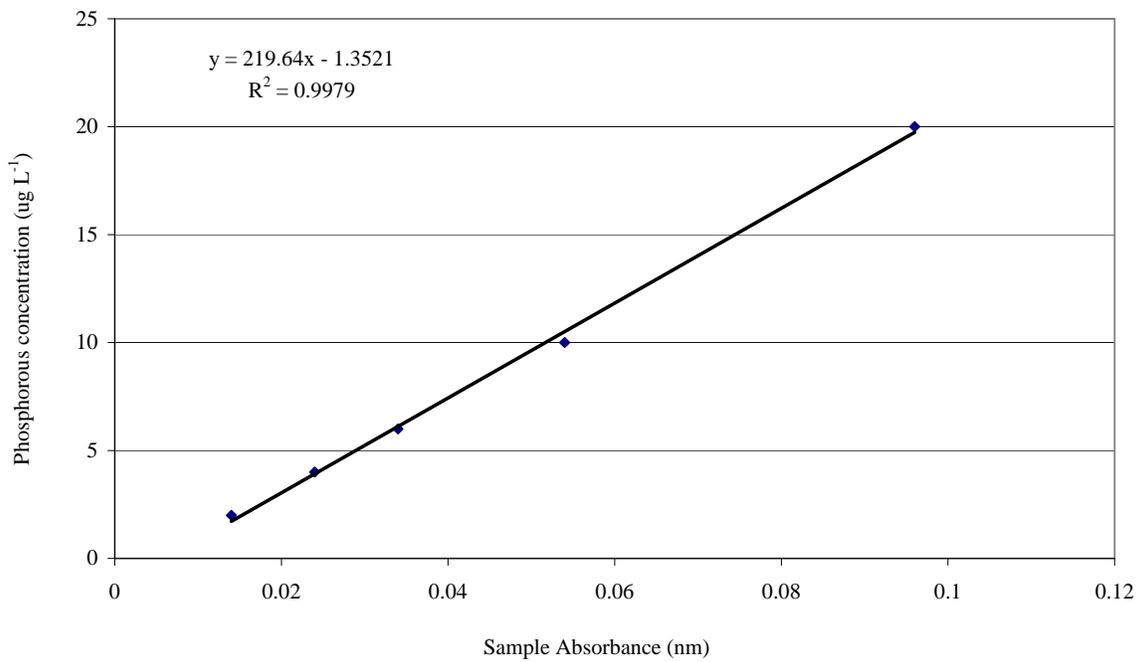


Figure 8.—Total phosphorous (TP) and total filterable phosphorous (TFP) concentration versus sample absorbance.

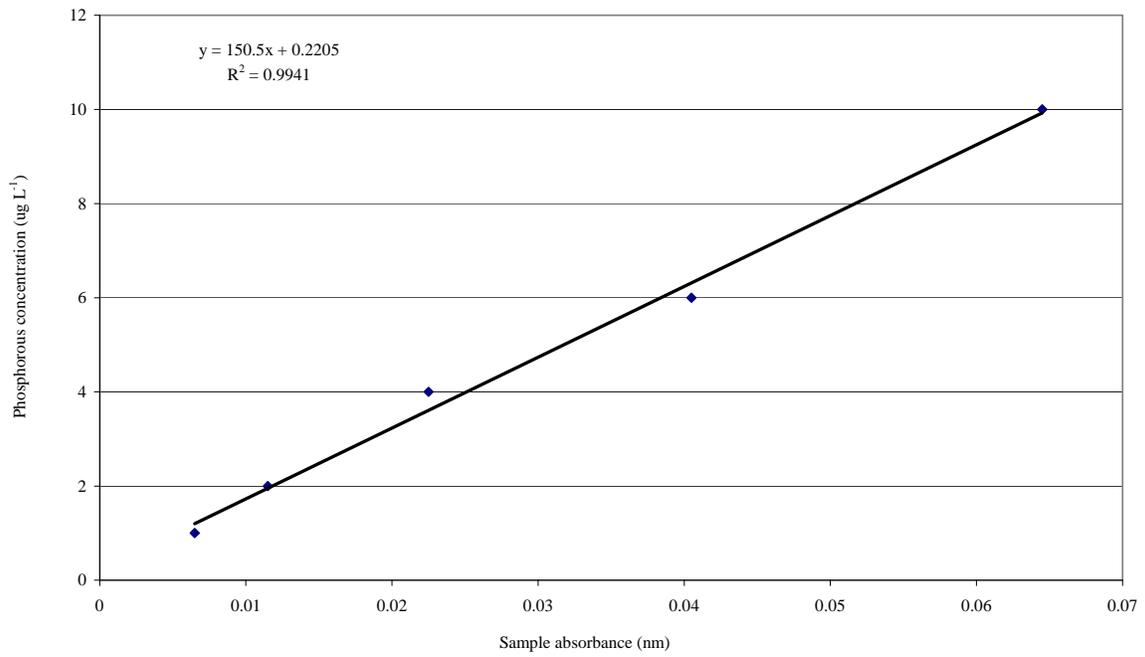
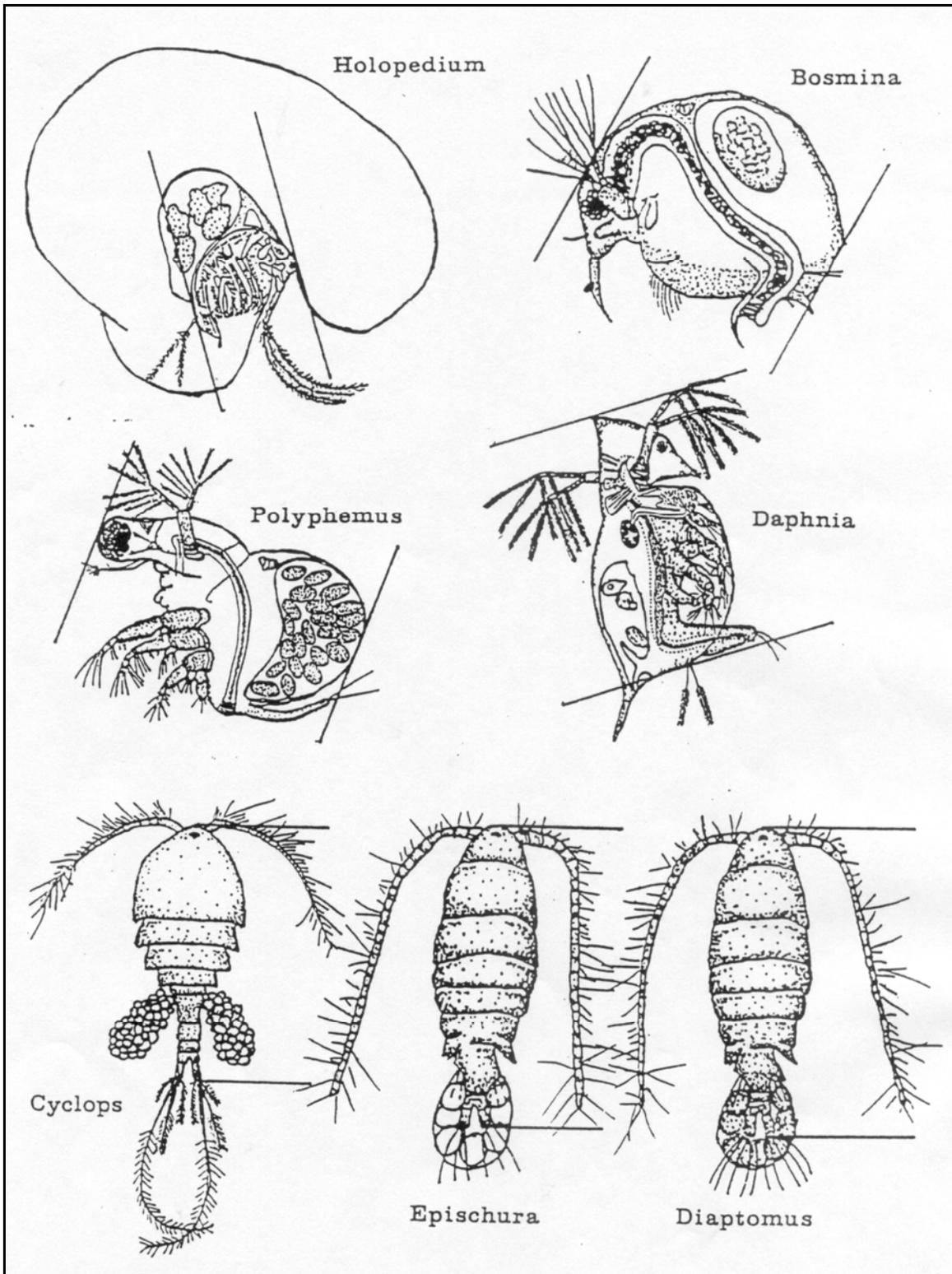
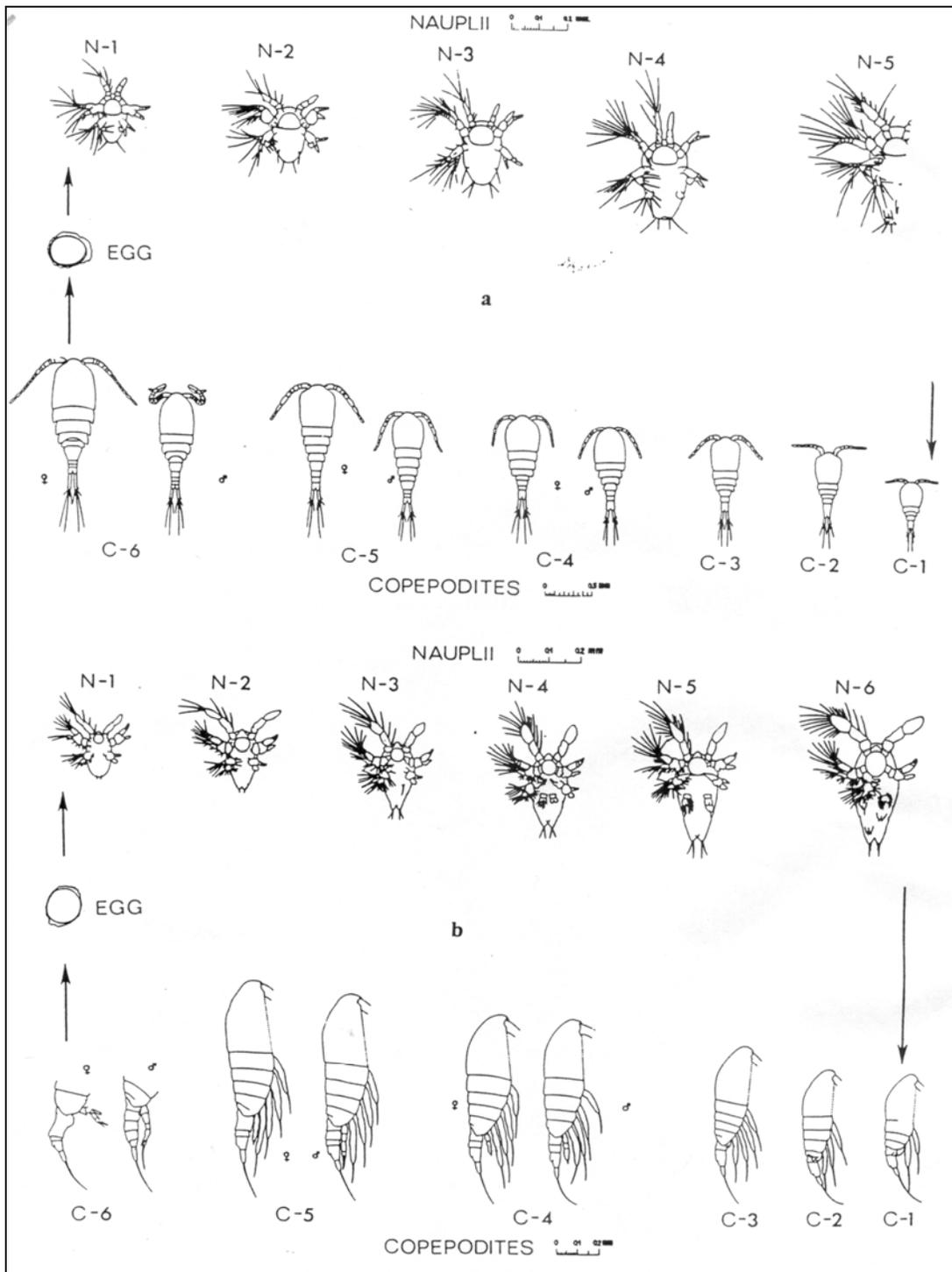


Figure 9.—Filterable reactive phosphorous (FRP) concentration **versus** sample absorbance.



(Koenings et al. 1987)

Figure 10.—Common cladocerans and copepods with locations of anterior and posterior measuring points.



(Wetzel and Likens 1991).

Figure 11.— Copepod life history stages.

APPENDIX A

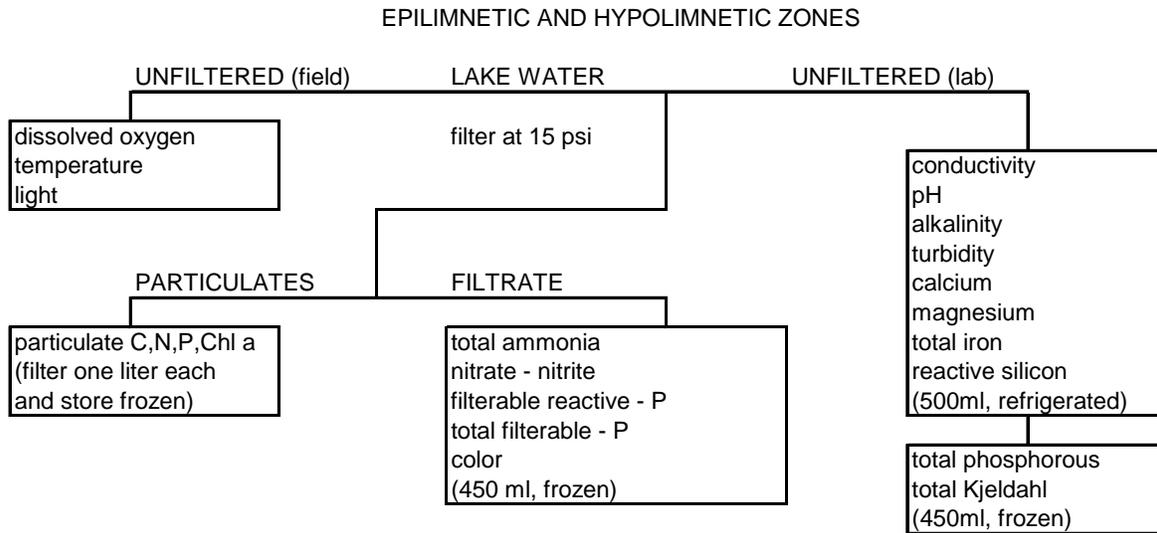
Appendix A1.–Field sampling equipment list.

Equipment	Task
4 L Van Dorn sampler	Water sampling
Reel with 55 m of rope marked in meters	"
Messenger	"
Carboys 1 8 L carboy for each station and depth	"
20-cm x 153-um mesh Plankton net with bucket	Zooplankton sampling
Reel with 55 m of rope marked in meters weight to test for bottom	"
1-125 ml polypropylene bottle (Per station) with 12.5 ml of concentrated buffered formalin in each bottle	"
D.O./temperature meter (cord marked in meters) membrane replacement kit	Temp/DO readings
Photometer (cord marked in 1/2 meters)	Light level readings
Secchi disk	Water clarity reading
Clip board, rite in rain DLS form, pencils	Data entry
Sampling box with hand held thermometer, DI water squirt bottle, knife, electrical tape	General sampling
Sandbags (3 bags per station) or anchor, Lake map, hand held GPS, rope and buoy.	Station set up only

Appendix A2.–Lab water filtering equipment list.

Equipment	Quantity / Size	Task
Polypropylene bottles (labeled) per sample carboy :	(2) 500 mL (1) 125 mL (1) 250 mL	1 for unfiltered refrigerate and 1 for filtrate frozen 1 for phytoplankton 1 for unfiltered. frozen
Whatman GF/F filters/petri slides per sample carboy:	(2) 4.25-cm	1 for particulate (C, N, or P) and 1 for Chlorophyll <i>a</i>
Graduated cylinders	(2) 1 L	Sample measurement
Filtering apparatus: (for 3 samples)	(6) filter tower (3) filtrate flask (2) vacuum pump vacuum hose (2) vacuum filter (1) Waste water flask	Particulate/Chloro filtering filtrate frozen collection
Wash bottle with DI water		Rinsing filter apparatus
Phosphate free detergent		Washing bottles, carboys, and glassware
Filtered water		Rinsing bottles, carboys, and glassware
Sterile forceps		Handling filter pads
Magnesium carbonate		Chlorophyll <i>a</i> preservation
Tin foil/parafilm		Covering filter apparatus

Appendix A3.—Sample collection protocol and procedures for lake water processing.



Adapted from Koenings et al. (1987).

Appendix A4.—Template 1 – Determination of sample number for adequate body size measurement.

Organism: _____ Lake: _____ date: _____

First 15 measurements

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15

n=

n-1=

t-statistic=

N=

N=number to be measured

mean
s.d.

Appendix A6.–Template 2 – Zooplankton count summary sheet.

Lake:											
Station:											
Depth:											
Date:											
Dilution:											
Counts											
	1	2	3	4	5	6	7	Mean	Total in sample	#/m ²	#/m ³
Ergasilis											
Epischura											
Ovig. Epischura											
Diaptomus											
Ovig Diaptomus											
Cyclops											
Ovig. Cyclops											
Harpacticus											
Nauplii											
Bosmina											
Ovig. Bosmina											
Daphnia l.											
Ovig. Daphnia l.											
Daphnia g.											
Holopedium											
Ovig. Holopedium											
Chydorinae											
Polyphemus											
Immature Cladocera											
Kellicottia											
Asplanchna											
Keratella											
Conochilus											
Filinia											
Ostracoda											
Egg thing											
Ceratium											
other rotifers											
	Body Size (mm)							Mean Body size (mm)			
Ergasilis											Ergasilis
Epischura											Epischura
ovig. Epishura											ovig. Epishura
Diaptomus											Diaptomus
ovig. Diaptomus											ovig. Diaptomus
Cyclops											Cyclops
ovig. Cyclops											ovig. Cyclops
Harpacticus											Harpacticus
Naupli											Naupli
Bosmina											Bosmina
ovig. Bosmina											ovig. Bosmina
Daphnia L.											Daphnia L.
ovig. Daphnia L.											ovig. Daphnia L.
Daphnia G											Daphnia G
Holopedium											Holopedium
ovig. Holopedium											ovig. Holopedium
Chydorinae											Chydorinae
Polyphemus											Polyphemus

Appendix A7.–Template 3 – Zooplankton density summary sheet by surface area.

Lake: _____
 Station: _____
 Year: _____

Macrozooplankton Density
 (no./m²)

Seasonal Mean
 (No/m²) group mean

Date:

- Ergasilis
- Epischura
- Ovig. Epischura
- Diaptomus
- Ovig Diaptomus
- Cyclops
- Ovig. Cyclops
- Harpaticus
- Nauplii

- Bosmina
- Ovig. Bosmina
- Daphnia l.
- Ovig. Daphnia l.
- Daphnia g.
- Holopedium
- Ovig. Holopedium
- Chydorinae
- Polyphemus
- Immature Cladocera

Sub Total

- Kellicottia
- Asplanchna
- Keratella
- Conochilus
- Filinia
- Ostracoda
- Ceratium
- other rotifers

Total:

Body Size (mm)

- Ergasilis
- Epischura
- Ovig. Epischura
- Diaptomus
- Ovig. Diaptomus
- Cyclops
- Ovig. Cyclops
- Harpaticus
- Nauplii

- Bosmina
- Ovig. Bosmina
- Daphnia l.
- Ovig. Daphnia l.
- Daphnia g.
- Holopedium
- Ovig. Holopedium
- Chydorinae
- Polyphemus

	Weighted Mean length (mm)	Weighted mean length (mm)	Biomass (mg/m ²)	Weighted Biomass (mg/m ²)	Group wt'd biomass (mg/m ²)	Group wt'd length (mm)
TOTAL:						

APPENDIX B

Appendix B1.—Apparatus used to process samples by vacuum filtration to obtain particulates and filtrate: A) using one filtrate flask and B) using a filtering manifold.

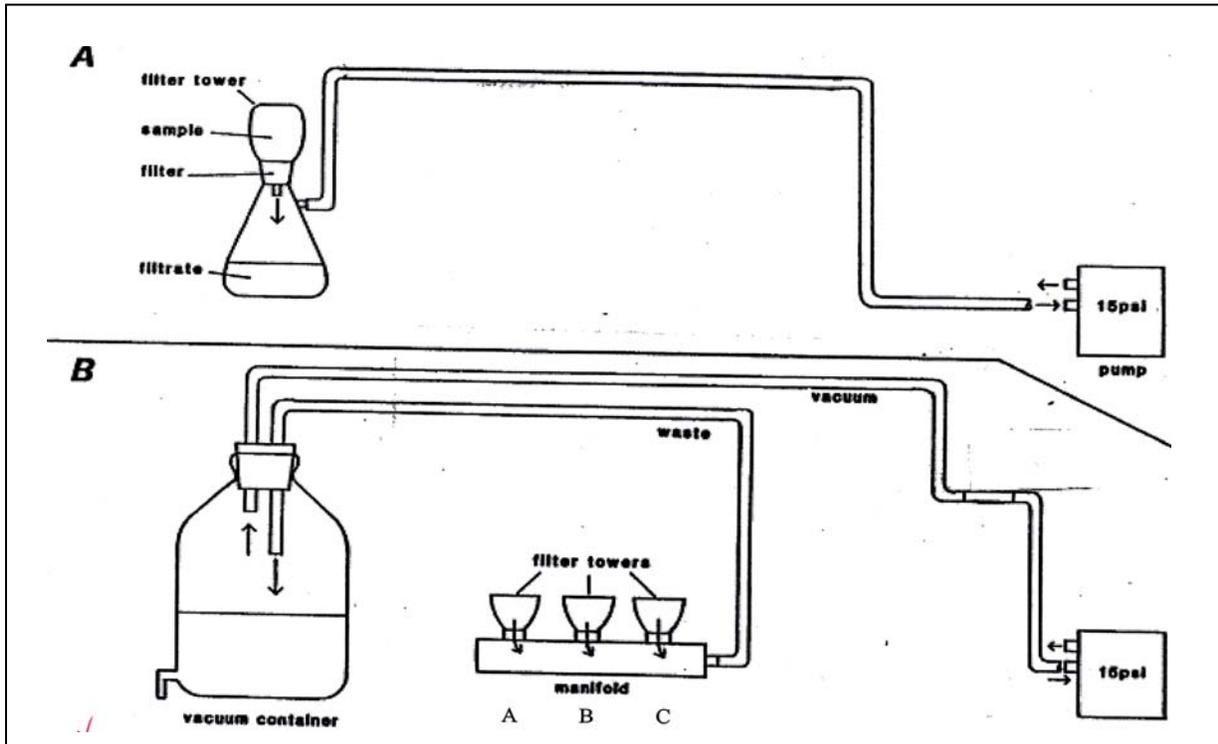


Figure adapted from Koenings et. al. (1987).