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LIMNOLOGY FIELD AND LABORATORY MANUAL: METHODS FOR ASSESSING AQUATIC PRODUCTION

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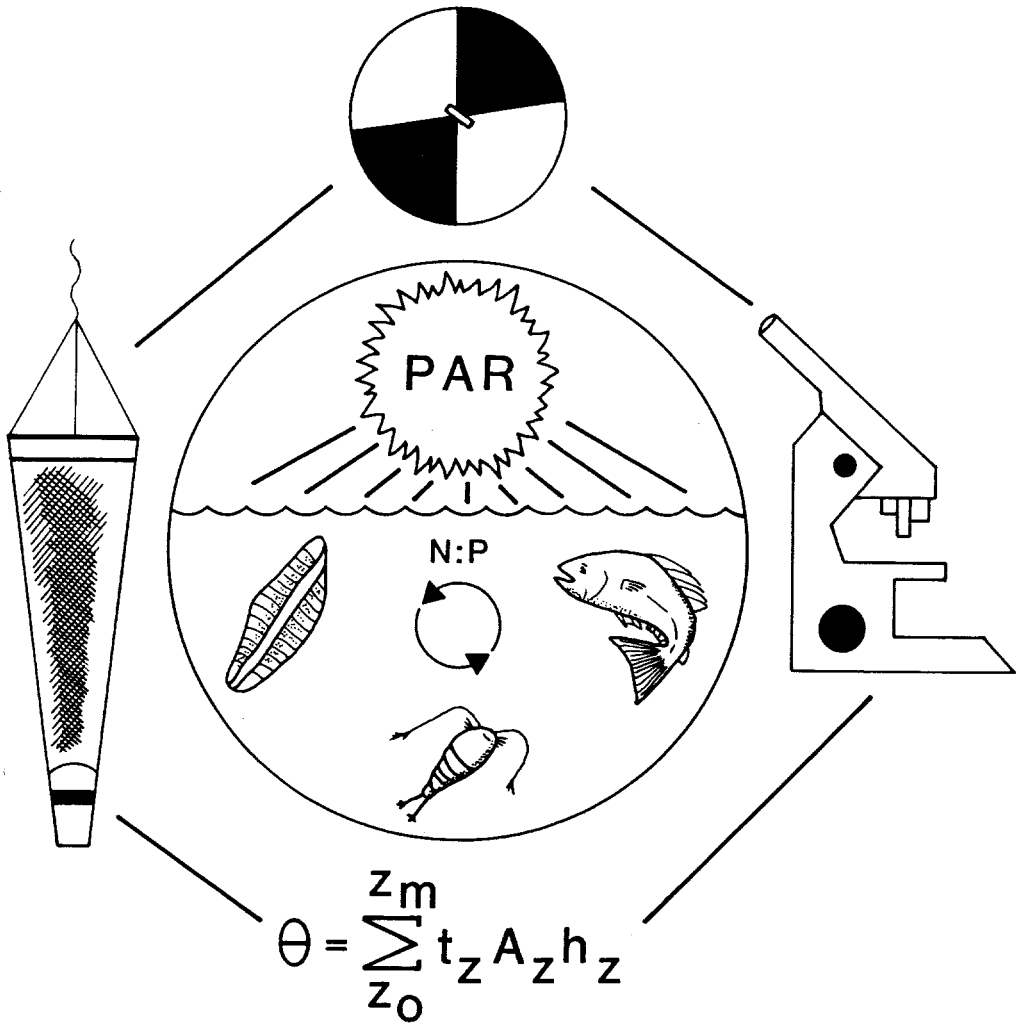
LIMNOLOGY FIELD & LABORATORY MANUAL



ALASKA DEPARTMENT OF FISH & GAME

Fisheries Rehabilitation, Enhancement & Development Division (F.R.E.D.)

Limnology Section

PREFACE

This manual describes the limnological field techniques and laboratory methods currently used by the Limnology Section of the Alaska Department of Fish and Game (ADF&G), Fisheries Rehabilitation, Enhancement, and Development (FRED) Division to assess aquatic production. In order to develop a comprehensive limnological data base for a specific lake system, its physical, biological, and chemical characteristics must be determined. Since the limnology program, which involves many geographically isolated personnel, is currently investigating lakes statewide, it became apparent that a concise reference describing the field and laboratory procedures should be made available as a guide in limnological research. Thus, the purpose of this manual is to provide statewide standardization of both laboratory and field procedures in order to insure consistency and quality in the collection and processing of aquatic samples.

Although the primary focus of the text is to describe the various procedures in use, brief introductions concerning the importance or relevance of each limnological parameter and principle have been included. Part I of the manual discusses the sampling procedures used for sample collection and storage, and for conducting field measurements. Part II constitutes the laboratory section, which describes the techniques and procedures for analyzing water quality samples, measuring primary productivity, enumerating and identifying zooplankton, and evaluating the food habits of juvenile salmon. Part III addresses instrument calibrations, and statistical evaluations of analytical methodologies.

This manual is designed to support the ADF&G, FRED Division, Lake Enrichment and Lake Stocking Programs as well as those of cooperative state, federal, and private agencies. The authors intent is to provide a comprehensive procedural reference for both field and laboratory personnel, as adoption of statewide standard methodologies will further the consistent interpretation of limnological results.

A preface permits the authors to express appreciation to those colleagues who have influenced the contents. Most conspicuous have been Robert Burkett and Paul Woods (U. S. Geological Survey) whose support and advise on the format and scientific content was much appreciated. The authors also thank Denise Cialek and Virginia Petanovitch for comments concerning autoanalyzer procedures, Richard Yanusz for suggestions on the chlorophyll a analysis and field procedures, and Dave Barto for the staff gauge information. Finally, the authors are extremely grateful to Carol Schneiderhan and Mary Whalen for the preparation of figures and illustrations and to Sue Howell for the seemingly endless task of typing this manual.

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INTRODUCTION

The alteration of watershed habitat, the stocking of salmonid fry, and/or the addition of nutrients to a lake system for the purpose of increasing the standing stock of salmon, impact all aspects of a lake ecosystem. Since few biological processes are determined by independently operating factors, such large-scale ecosystem manipulations become very complex and produce many changes within a lake. This is because a lake system can be described as a complex mosaic of interacting pathways joined by an array of complex yet concrete compartments (Figure 1). It is the innate complexity of these metabolic pathways that need to be understood in order for a lake environment to either be maintained or manipulated. Each choice demands knowledge, for without such understanding, lake management would be impossible.

Lake productivity and production are both dependent on the relative interaction of physical, biological, and chemical features operating within a given system. The combination of standing crop (compartments) and linkages (R) between them can be thought of as a 'fingerprint' of a particular system (Figure 1) and, thus, are in themselves unique. However, the driving mechanisms of lake production are ultimately linked to energy supply (sunlight) and the seasonal cycling of nutrients (N_{1-5}). Nutrient levels (e.g., phosphorus and nitrogen) fluctuate in availability over the course of a year and can become depleted when demand (R_2) exceeds supply (R_1). During these periods, generally over the summer, the production of phytoplankton is reduced and/or the production is lost (R_7) through nutrient utilization by nuisance groups of phytoplankton. Either change is felt first by the zooplankton, which directly feed (R_3) on specific phytoplankton groups, and then by successive tiers in the food chain, leading to a decrease in forage production (R_4) for rearing salmon fry. The point where fish-forage production (R_3) is exceeded by demand (R_4) is the capacity point of a lake, in terms of successfully rearing salmon fry, and defines rearing limitation of fish production.

Since the productive capacity of lakes, in terms of fish-forage production, is independent of the capacity of the same lake system to recruit rearing fry, specific lakes contain few, if any, salmonid fry. In these systems either the actual spawning area, the number of spawning adults (escapement), or both are low. Here fish-forage production (R_3) can exceed demand (R_4), and such lake systems define a recruitment limitation to fish production.

In-lake manipulation techniques (lake enrichment or lake stocking) are designed to either increase preexisting forage production (R_3) to satisfy the demands of rearing fry (R_4) or to increase the demand (R_4) on a high preexisting forage supply (R_3), respectively. That is, both techniques seek to balance

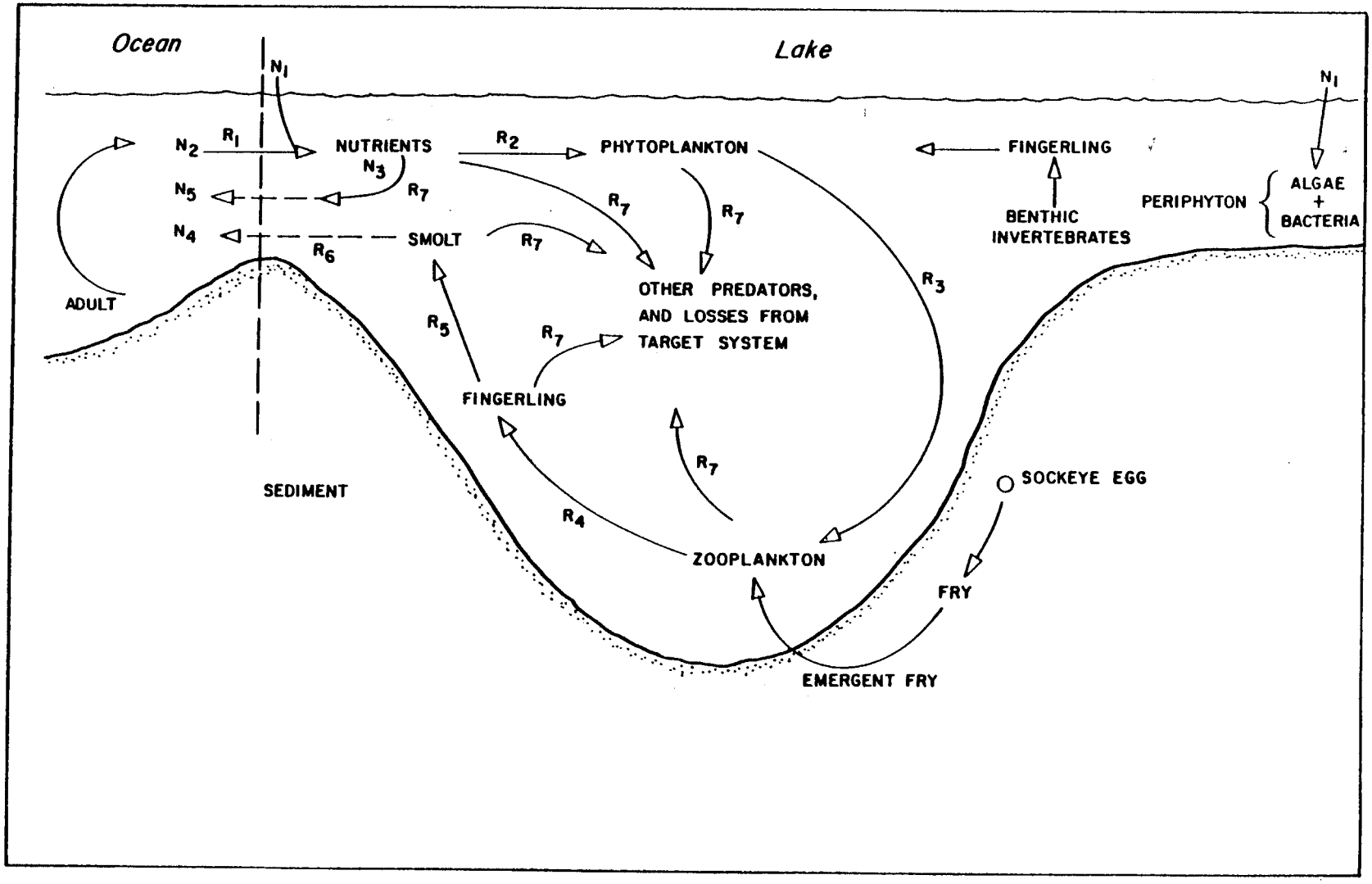

Figure 1. Generalized schematic of a lake ecosystem emphasizing metabolic pathways or linkages between trophic levels (see legend for detailed description).

N_1: Nutrient supply from the atmosphere, land runoff, and lake tributaries.

N_2: Nutrients imported in spawning anadromous fish.

N_3: Existing supply of available nutrients in the euphotic zone.

N_4: Nutrients exported from the system in outmigrant anadromous fish.

N_5: Nutrients lost to the system in the river outflow.

Phyto: Standing crop of primary producers, the phytoplankton.

Zoopl: Standing crop of secondary producers, the zooplankton. Note that this element in the food chain is instrumental in the linkage between emergent fry and rearing juvenile. If artificial nutrients are applied, they must be introduced at N_1, so that zooplankton populations are synchronized (species, timing, and location) to entry of fry into limnetic rearing areas. If this is not done properly, food chains might be stimulated that may have a detrimental effect on targeted salmon or trout production.

Competitors: Includes non-target species that may be competing for food organisms. Examples are stickleback, white fish, etc.; this category may also include littoral communities (also in competition for food supplies) utilizing lake nutrient supplies (N_3).

For example, N_3---➔ periphyton ---➔ snail.

Rearing Juvenile: Standing crop of rearing sockeye juveniles (modifications can be made for other species).

Smolt: Production from the lake represented in the outmigrant target species of fish. This could be thought of as a final product of a lake fertilization project and, as ocean survival of smolts is related to size (length), one system may respond to a strategy of few large smolt while another system may respond to the strategy of many smaller smolts.

Adult: The standing crop or biomass accrued outside of the system. Increase in this standing crop occurs by way of energy and nutrient sources in the ocean. Returning adults (spawners) may be an important source of nutrients for the lake system.

R_1: Rate of nutrient input to the lake system. Includes atmospheric and watershed inputs as well as nutrients supplied in carcasses of returning adult salmon.

R_2: Rate of nutrient uptake from dissolved available forms in the water column to biomass as primary producers. This uptake rate is measured in minutes, and is reflected as biomass of primary producer in 4 or 5 days.

R_3: Rate of biomass change from primary to secondary producer. This linkage of the food chain is the energy source for production of rearing fish forage. It must be of proper species composition in the proper location at the appropriate time. Interacting factors necessary to achieve this may be critical e.g., temperature, and water clarity.

R_4: Rate of biomass change for the rearing phase of lake populations of salmonids. Acceptable growth of rearing fish populations depends upon a continuous supply of food organisms. Again, for biomass to appear as the desired salmonid linkage proper species and location must be accomplished through judicious application of fertilizer at the N_1 phase.

$R_{5,6}$: Rates of change to smolt outmigrant biomass as a result of the survival and growth of the rearing salmonids. Outmigrating smolts represent the desired end product of the freshwater food chain.

R_7: Rate of change of biomass to non-target elements within the lake food web. For example, phytoplankton can be cropped by zooplankton that are not appropriate as salmon food, competitor or predator species of fish can crop zooplankton or rearing salmonids; and nutrients can be side channeled and bound into macrophytes.

Such side channels from the desired food chain can produce serious losses to lake fertilization programs, and may reduce the benefit/cost ratio below feasible levels.

Summary: Energy and nutrient flow in a lake is a product of nutrient supplies and the factors of sunlight, temperature, and the nature of the lake basin. Successful production of targeted species will be enhanced by minimizing biomass side channels (R_7) such as large littoral communities and large populations of predator/competitor species. Other needs of the target species, such as spawning area, should be considered.

Successfully managed lake fertilization or fry stocking projects will have low standing crop levels and high rates of change with biomass moving rapidly through the food chain to support growth of targeted fish populations. Little or no change should be detectable in the other elements within the lake's ecosystem.

the delivery of fry with the ability of a lake to produce forage as a way to optimize (reduce R_7) the use of a given carrying capacity. However, the application of the proper enhancement technique requires prior knowledge of where a lake lies, relative to the balance point. The ranking of each lake, relative to that point, can be assigned, given an understanding of in-lake indicators, which for example, reflect the relative magnitude of fry-feeding pressure on the zooplankton community.

Through a basic understanding of how production linkages are made and what the key indicator variables are, empirical models can be fabricated (Koenings and Burkett 1986a, 1986b; Koenings et al. 1986). Through the use of such models, management of lake systems for a given product can be undertaken with increased likelihood of success. The field and laboratory procedures described herein represent the latest methods and techniques for comprehensive lake inventories or research-project evaluations. It is our intent to provide a basis for standardized limnological assessment in order to study rearing-fry ecology through an understanding of lake trophic-level dynamics.

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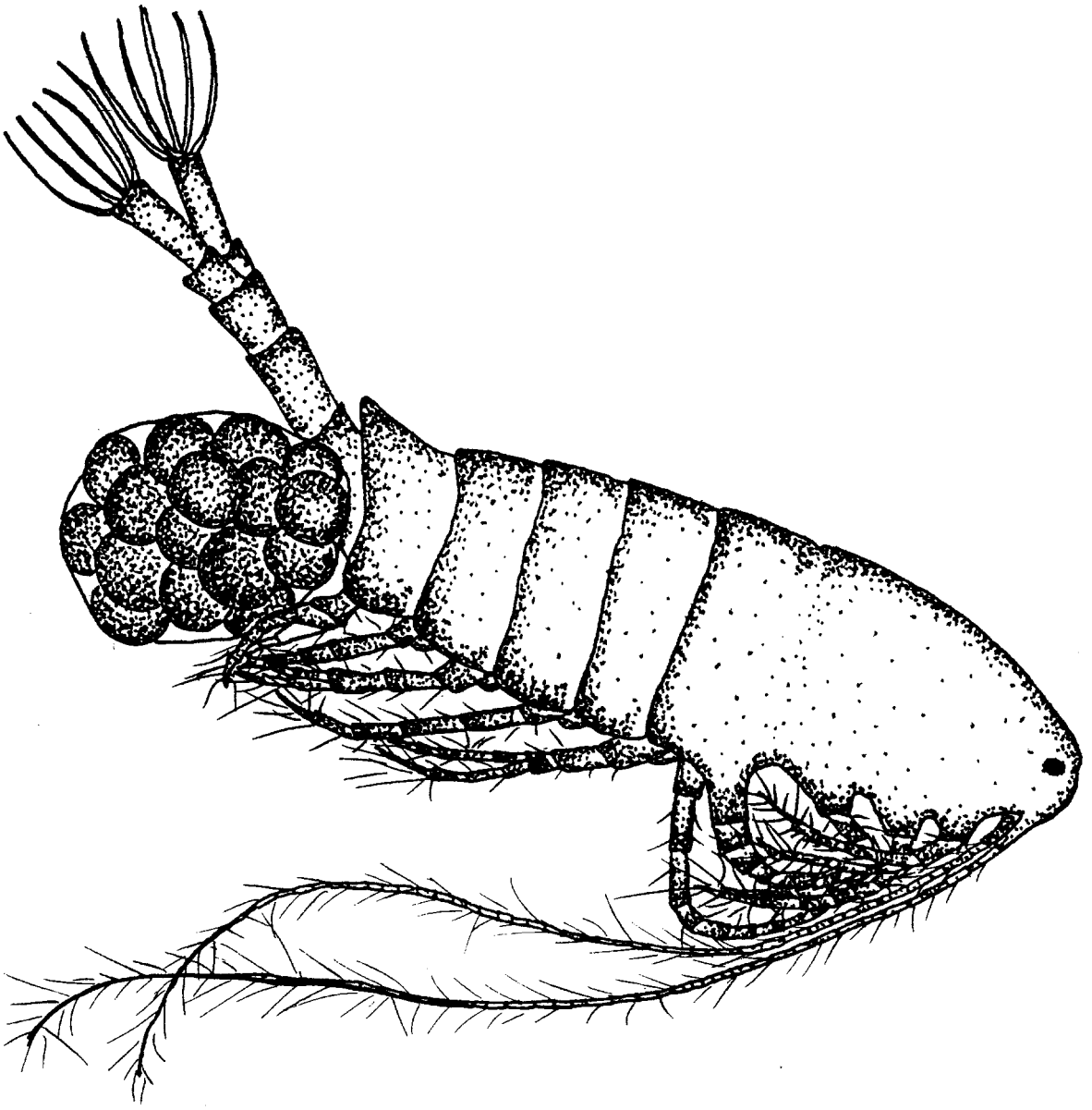
PART I.

SAMPLE COLLECTION TECHNIQUES AND FIELD MEASUREMENTS



SAMPLE COLLECTION AND STORAGE

Cleaning Glassware and Plastics

Three grades of water are used in the limnology laboratory: Type I, Type II, and distilled water. The primary water-purification system is a Milli RO-20 system equipped with an additional Milli Q reagent grade water filter.

Type I Water (DI) - Type I or de-ionized (DI) water has been filtered through a series of ion exchange cartridges of the Milli Q system after reverse osmosis. The specific conductance is less than 1.4 umhos/cm. All reagents and standards are prepared using DI water.

Type II Water - Type II is tap water that has been purified by the process of reverse osmosis (RO) using the Milli RO-20 system, and stored in an epoxy-lined, aluminum reservoir. RO water has a specific conductance of less than 40 umhos/cm.

Distilled Water - Distilled (DW) water is tap water purified by distillation using a Corning Mega Pure still. Freshly distilled water has a specific conductance of <2.0 umhos/cm, but will increase to 2-4 umhos/cm within 48 hours.

Clean all glassware and plasticware, including nutrient sample bottles, prior to use according to the following procedure:

1) Wash glassware and plastics with phosphate-free detergent and rinse with tap water.

2) Rinse with 10% hydrochloric acid (HCl).

3) Rinse four time with Type II water.

4) Rinse twice with DI water.

Carboys - Wash large containers with phosphate-free detergent and rinse three times with DI water.

Zooplankton bottles - Wash bottles with phosphate-free detergent and rinse with tap water. Bottles used for zooplankton samples shoud not be re-used for nutrient samples.

Particulate nutrient filters - Clean Whatman GF/F filters by placing on the filtering apparatus and drawing 50 ml of DI water through the filter. Clean circular or funnel filters by rinsing with DI water using a wash bottle. Always use blunted forceps when handling filters, and avoid using 10% HCl to rinse filters.

General Sampling Protocol

In order to collect representative field data, maintain data consistency, and avoid sample contamination, there are several considerations to bear in mind. Routine field-sampling techniques conducted at each lake station involve collection of water samples, vertical zooplankton tows, measurements of temperature, dissolved oxygen, light penetration, pH, and Secchi-disk depth, and performing in-situ experiments with carbon-14 to estimate primary productivity.

The establishment of permanent station markers is necessary in order to conduct limnological sampling at the same location over time. This is crucial, if changes in water quality are to be accurately monitored and interpreted with confidence; because at any one instance differences in chemical, biological, and physical conditions may exist at different areas within a lake. In addition, lake processes may vary considerably over time. At least two sampling stations per lake are recommended; however, several stations may be required to fully characterize a lake system consisting of several basins or bays.

To closely monitor lake dynamics, field sampling should be conducted approximately every three weeks during the ice-off to ice-on period and once during mid-winter (ice cover) throughout intensive sampling; e.g., prefertilization project phase. During the less intensive feasibility phase, the same field work is conducted less frequently, usually four times a year (spring turnover, summer stratification, fall turnover, and mid-winter).

The two most recurrent problems affecting water sample collection are contamination and the delay in the initial sample processing (filtering). By adhering to a few simple procedures these problems can be eliminated. Prior to sample collection make sure all carboys are cleaned (see Cleaning Glassware and Plastics, p. 6). Once at the sampling site, rinse the carboys with sample water and discard before storing the actual sample. The rinsing process permits 'aging' of the vessel walls and prevents any subsequent loss of chemical constituents from the water to the polyethylene (poly) surface. Keep the carboys and sampling gear away from all petroleum products. Containers or sample bottles contaminated by outboard fuel, gasoline, or oil are impossible to clean, and must be discarded.

Keep all field equipment clean, dry, and fully charged. An important note to remember is that battery power is dependent on temperature; i.e., because an instrument runs well at 20 C does not mean it will function properly at 0 C. Calibrations should be conducted at each sampling station to insure the accuracy of the measurements.

An additional point worth mentioning concerns communication between field personnel and the laboratory. Sample analyses are useless without complete and proper documentation. Therefore, label all bottles and complete the inventory forms prior to sending them to the laboratory. Moreover, if in doubt concerning a meter reading, depth of sample, sample label, etc., repeat the procedure. A little time lost at this point is far better than trying to either decipher unclear notes or interpret faulty data months later.

Finally, during sampling record general lake conditions. These include weather observations, such as temperature, cloud cover, precipitation, and wind speed as well as notes on lake surface conditions, water color, ice depth, period of ice cover, etc. These incidental notes may prove beneficial when examining the field data at a later date.

Lake Water

Using either a Van Dorn sampler or Kemmerer bottle (Figure 2), collect water samples from each sampling site at the 1.0-m depth and the mid-hypolimnion. If the thermocline is absent, take the deeper sample from 75% of the lake depth. Collect 5 liters of water for each sample and pour it directly into a clean carboy or other poly-container. Remember to rinse out the carboy with sample water (~1 liter obtained from the first cast) prior to sample collection. Discard any samples containing sediment, and resample a few meters higher.

Keep the carboys (samples) cool (4 C) and in the dark, at least out of direct sunlight, until they can be processed. In turn, during winter sampling keep the samples from freezing. It is important to filter the samples as soon as possible following their collection. Delaying the filtering beyond 3-4 hours is undesirable because of gross changes that may occur in the chemical composition of the sample.

Dissolved Gases

Collect a water sample using a Van Dorn bottle with a length (~10 cm) of tygon tubing attached to the outlet drain. Place the tube outlet to the bottom of a 300-ml BOD bottle, and open the valve, allowing the bottle to overflow. Carefully insert the stopper without leaving an airspace, store in the dark at 4 C, and analyze as soon as possible.

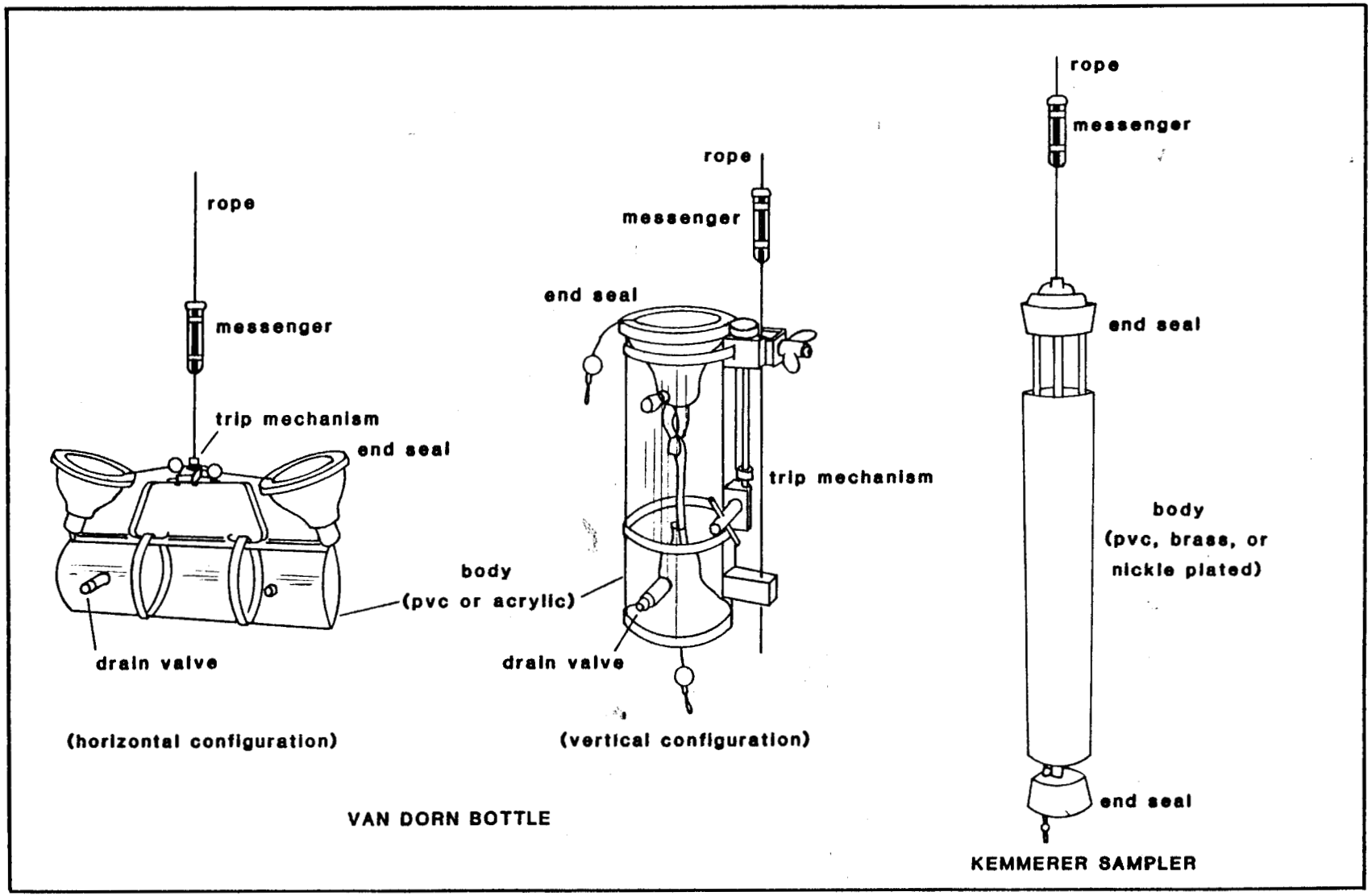


Figure 2. Water collection bottles used to sample specific depths in the water column without contamination from overlying waters.

Periphyton

Suspend sets (2) of plexiglass plates (10 x 10 cm is suitable) at the 1-m depth, and above a stable subtrate within the littoral zone. After initial colonization and growth (~3-6 weeks), scrape the periphyton from both sides of each plate into a funnel, and rinse the contents with tap water into a 250-ml polybottle. Store at 4 C in the dark, and return the sample as soon as possible to the laboratory. Reset the plates for subsequent sampling at 3-6 week intervals throughout the sampling season.

NOTE: Rough the surface of new plexiglass plates with steel wool, and soak in tap water for several days to 'age' prior to use.

Plankton

For phytoplankton identification and counting; use water collected from the 1-m depth, as described above, during spring turnover, summer stratification, fall turnover, and mid-winter. Pour 100 ml of unfiltered lake water into a 125-ml polybottle.

For zooplankton identification and counting, use a 20-cm diameter by 153-um mesh net for clear-water lakes or systems with high zooplankton densities. Use a 0.5-m diameter net for glacial lakes or systems with low zooplankton densities. Collect two replicate tows from each sampling station. Lower the net to 50 m or just off the bottom if the lake depth is less than 50 m. Pull the net vertically to the lake surface at a rate of 0.5 m/sec. Rinse the net by immersing several times in the lake to just below the net collar. This washes organisms caught in the mesh into the net bucket. Remove the bucket from the net and using a wash bottle, rinse the contents into a 125-ml polybottle. Label with the following information: lake, station, date and time, depth of tow, tow number, and type of net; i.e., diameter and mesh size.

Stream Water

Generally 500 ml of unfiltered water are required for stream samples. Collect the sample by submerging a 500-ml polybottle upstream from where anyone has crossed or waded. Rinse the bottle with sample before storing in the dark at 4 C.

Sample Preparation

Lake water contained in carboys, is processed (filtered) and stored according to the type(s) of analysis to be conducted; unfiltered refrigerated, unfiltered frozen, and filtrate frozen water are saved for each sample (Figure 3). The filtering procedure and a synopsis of samples to be returned to the limnology laboratory are described below.

Clean all filter flasks, towers, and other necessary labware (see Cleaning Glassware and Plastics, p. 6). A vacuum pump capable of 15 psi negative pressure and sufficient to filter at least 4 subsamples simultaneously is necessary. If the apparatus is assembled as in Figure 4A, use one flask for the filtrate, and a separate flask for filtering chlorophyll *a* samples. If the apparatus is assembled as in Figure 4B use one filter tower for all chlorophyll *a* samples, and the single filter flask for filtrate. To avoid contamination, conduct all the filtering and storage of one sample and rinse the filter towers and filtrate flask with DI water before continuing to the next.

1) Particulate nutrients - Place a clean 4.25-cm GF/F (particle retention size of 0.7 um) Whatman filter on the base of the filtering apparatus. Filter one liter of lake water (if possible) at $\leq$15 psi using separate filters for each particulate carbon (C), nitrogen (N), and phosphorus (P) sample. Place each filter in a separate petri-slide, label (including the volume filtered), and store frozen.

2) Chlorophyll *a* (chl *a*) - Follow the same procedure used for particulate nutrients; however, as the last 50 ml of sample is filtered, add ~5 ml of $MgCO_3$ solution to the filter. This prevents acidification of the sample and subsequent conversion of chl *a* to phaeophytin (phaeo *a*).

3) Unfiltered refrigerated - After rinsing a clean polybottle with a small amount of sample, pour 500 ml from the carboy directly into a 500-ml polybottle without leaving an airspace. Store the sample in the dark at 4 C.

4) Unfiltered frozen - After rinsing a clean polybottle with a small amount of sample, pour ~200 ml from the carboy directly into a 250-ml polybottle and store frozen. Remember to leave an airspace within the bottle to allow for expansion of the freezing water.

5) Filtrate frozen - Filter ~100 ml of the sample to age the filter flask, and discard. Filter the remaining ~900 ml of sample retaining both the particulate nutrient filter

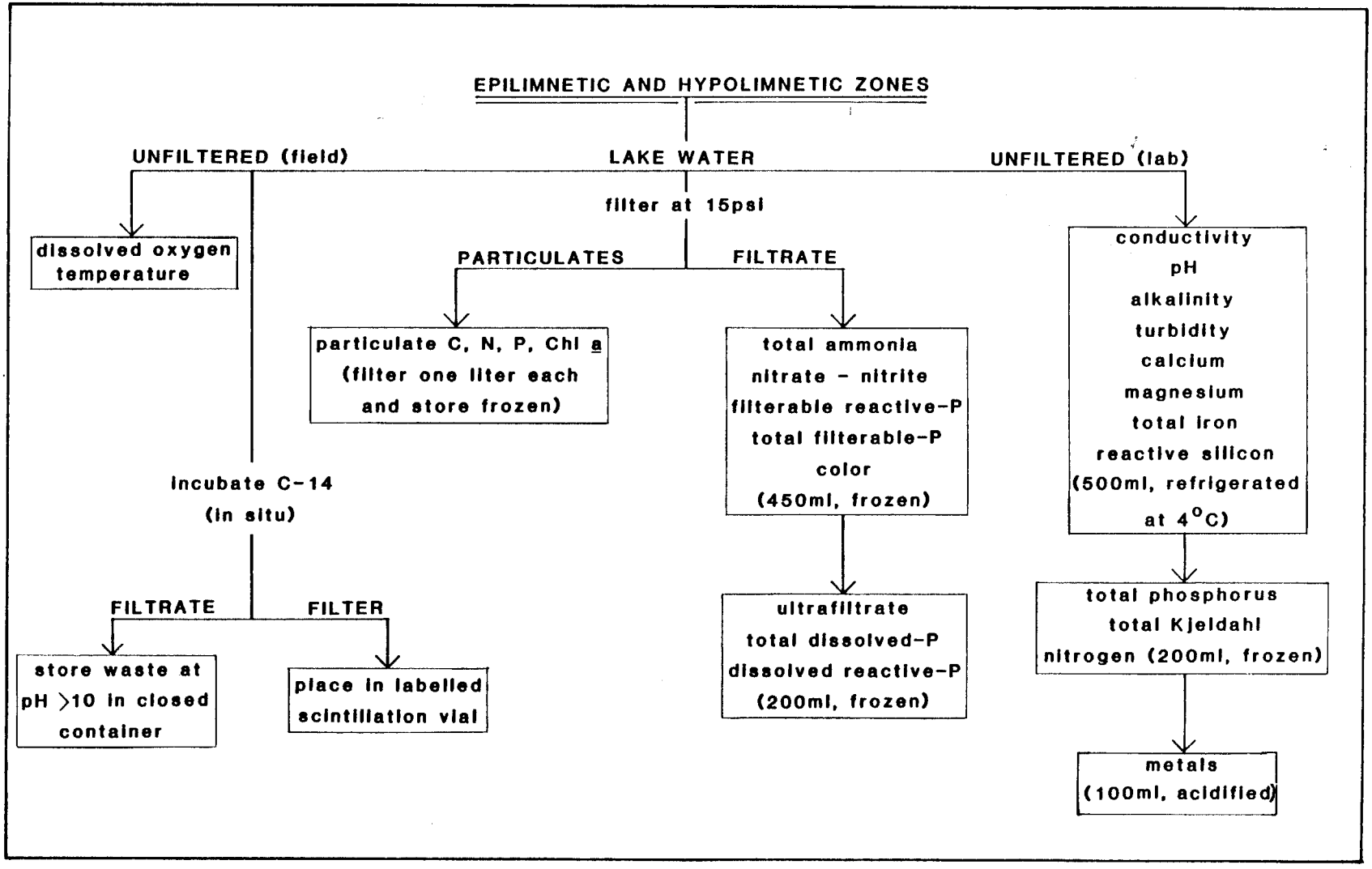


Figure 3. Sample collection protocol for field measurements and incubations; and procedures for lake water processing emphasizing three fractions, unfiltered, filtrate, and particulate. Also shown are the analysis conducted on each fraction and the recommended storage procedures.

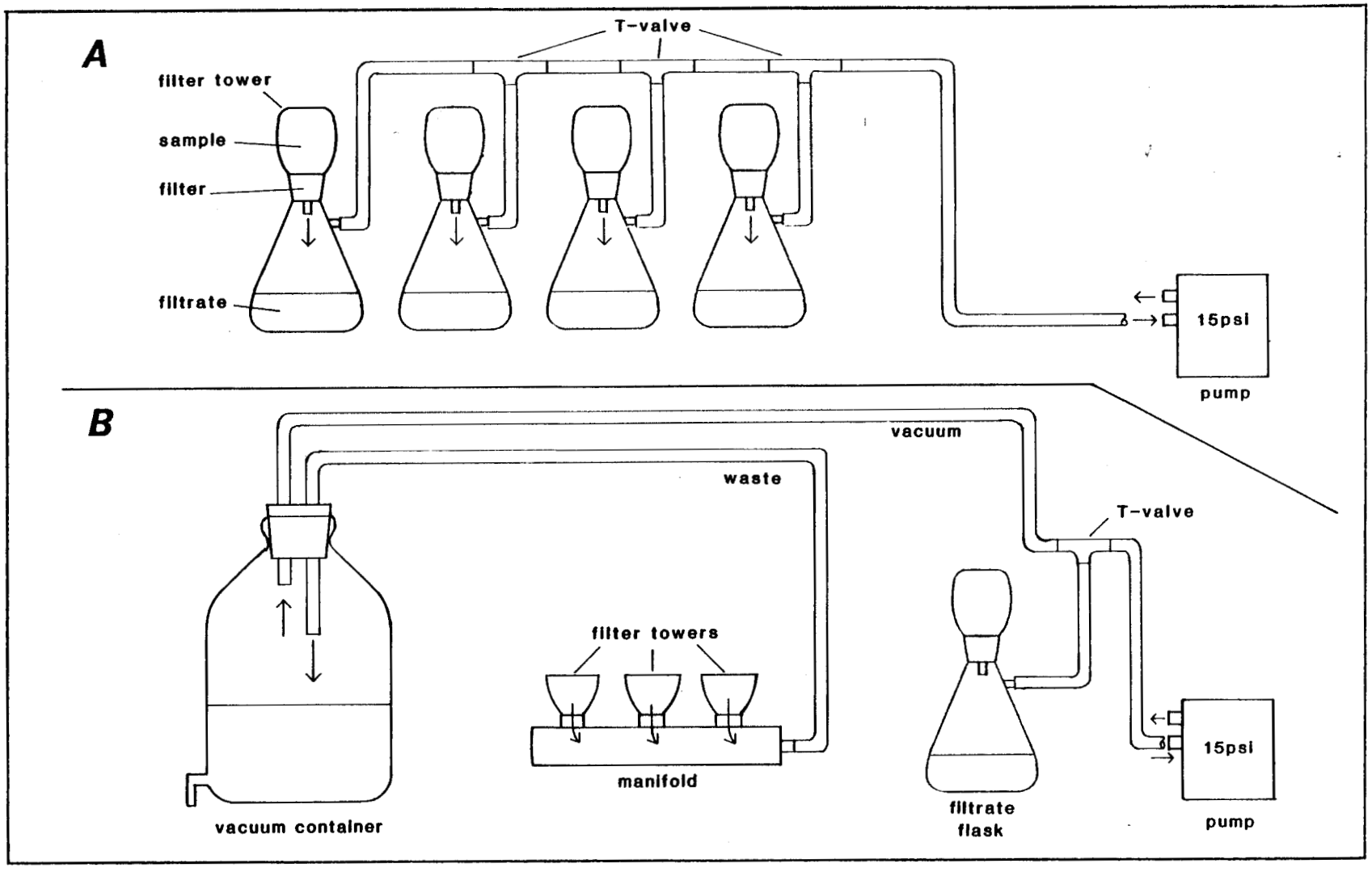


Figure 4. Apparatus used to process samples by vacuum filtration to obtain particulates and filtrate (A) using four separate filtrate flasks with individual towers, and (B) using a filtering manifold and a single filtrate flask.

and filtrate. Pour ~450 ml of filtrate into a 500-ml polybottle and store frozen.

NOTE: Remember to rinse all sample bottles with a portion of the actual sample prior to storage, and fill out all bottle labels completely.

Summarizing, to guarantee a complete water-quality analysis, each carboy should yield the following samples, to be sent as quickly as possible to the laboratory, for analysis (Figure 3).

1) 500 ml unfiltered refrigerated.
2) 200 ml unfiltered frozen.
3) 450 ml filtrate frozen.
4) 3 filters (1 liter each) for particulate C, N, and P.
5) 1 filter (1 liter) for chl *a* and phaeo *a*.

Alternately, our recent use of autoanalyzers reduces the sample volume necessary for certain tests, and the use of the fluorometric method for chl *a* reduces the volume of sample needed on a filter. The following volumes can be used; but may not allow retesting of inconsistent replicates.

1) 250 ml unfiltered refrigerated.
2) 200 ml unfiltered frozen.
3) 225 ml filtrate frozen.
4) 3 filters (0.5 liter each) for particulate C, N, and P.
5) 1 filter (0.5 to 0.25 liters) for chlorophyll *a* and phaeophytin *a*.

Metals

Collect samples for metal analysis twice a year: once before lake stratification in the spring and once prior to fall turnover. Pour ~100 ml of unfiltered water into a 125-ml polybottle, add 0.5 ml of 1:1 nitric acid, and store at room temperature.

Periphyton

Filter periphyton samples according to the procedure described for chl *a* sample preparation (p. 11). Record the area of the plexiglass plates on the petri-slide label.

Phytoplankton

Add 2.0 ml of Lugol's acetate solution to 100 ml of unfiltered water in a 125-ml polybottle, and store at room temperature.

Zooplankton

Preserve samples with 100% neutralized formalin (37% formaldehyde) to make a final 10% volume/volume formalin/sample solution (equivalent to a 3-4% neutralized formaldehyde solution). Alternately, use a wash bottle containing 10% neutralized formalin to rinse zooplankters from the collection net into a 125-ml polybottle. Store at room temperature.

Preservatives for Sample Storage

1) Magnesium carbonate ($MgCO_3$) - Mix 1 g of magnesium carbonate-n-hydrate in 100 ml of DI water. Mix thoroughly just before adding to the chl *a* samples because the $MgCO_3$ does not dissolve.

2) Lugol's acetate - Dissolve 10 g of potassium iodide, 5 g of iodine, and 5 g of sodium acetate-trihydrate in 70 ml of DI water.

3) 10% Neutralized formalin - Mix one part 100% formalin (37% formaldehyde) with nine parts of DI water. This yields a 10% formalin or 3.7% formaldehyde solution. Neutralize with 1 N sodium hydroxide (~1-2 ml per liter) until a pH of 7-8 units is reached. As NaOH is usually supplied as pellets, add ~1 pellet per liter of 10% formalin.

4) Nitric acid (1:1) - Carefully and slowly add one part concentrated nitric acid to one part DI water.

FIELD MEASUREMENTS AND APPLICATIONS

Temperature

The pattern of heat intensity within a lake (as measured by temperature) influences chemical reactions, nutrient cycles, and, ultimately, productivity. Thus, temperature is one of the most important physical aspects of an aquatic ecosystem.

In most lakes the increasing air temperatures and sunlight of spring begin to warm the surface layers forming the epilimnion. With increasing depth, the water column undergoes a rapid decrease in temperature (>1 °C/m) forming a thermocline or metalimnion which separates the colder layer below or hypolimnion. When these discrete layers form, due to differing densities, a lake is stratified and little or no mixing occurs between layers. In some deep oligotrophic lakes, a well defined thermocline may be absent, and instead, a gradual warming of the upper water column occurs. During the fall, which is accompanied by decreasing ambient air temperatures and increasing winds, the epilimnion gradually cools to the temperature of the hypolimnion. Eventually the entire water column achieves a uniform temperature (isothermy), and the water column slowly begins to mix from top to bottom (turnover). This is one of the processes by which nutrients are redistributed throughout the lake.

Procedure

Before taking temperature measurements, calibrate the thermometer by placing the probe in a mixture of ice and water. The ice-water mixture is 0 C. Record the temperature to the nearest 0.5 C at 1 meter intervals when the lake is stratified by lowering a 50-m cabled probe through the water column, and at every other meter when the lake is isothermal.

Lake-Heat Budgets

In-lake temperatures can be used to calculate heat budgets providing comparisons of thermal capacities and temperature regimes among lakes. We are primarily concerned with the summer heat budget because juvenile salmon growth is limited at temperatures below 4 C. The summer heat budget is defined as the accumulated calories (stored heat) within the lake from isothermy at 4 C in the spring to the summer maximum temperature. It is determined by both heat intensity and the duration of the heating period. In general, the heat budget is calculated after Birge (1915) by integrating the product of temperature and the volume of water at selected depth intervals.

Calculations

1) Construct a plot of lake temperatures (Y-axis) using 4 C as the origin versus the duration (days) of the growing season (X-axis). The growing season is the number of days above 4 C in the spring to isothermy at 4 C in the fall. For each sampling date, plot the temperatures from a minimum of 5 depth increments, beginning with the 1-m strata, and continue to the lake bottom or to 4 C. Draw a smooth curve through the points (Figure 5) forming five depth contours.

2) Using a calibrated planimeter (see Instrument Calibrations, p. 192), measure the area beneath each depth-contour from 4 C in the spring to the maximum temperature. Convert the planimeter reading to the number of degree-days (Table 1a).

3) Divide the number of degree-days by the number of days from 4 C in the spring to the summer maximum temperature to determine the mean temperature of each depth increment (Table 1a).

4) Use the mean temperature of an upper increment to represent each depth interval. For example, the 5-m mean temperature represents the 5 to 10-m depth interval (Figure 5). Multiply the volume of water (ml) contained within each depth interval by the mean temperature to determine ml- C (Table 1b).

5) Sum the results for each depth interval to obtain the total accumulated ml-C (Table 1b). As one calorie is required to raise 1 g (1 ml) of water by 1 degree, ml- C is equivalent to gram-calories (g-cal).

6) Divide the total number of g-cal by lake surface area (cm^2) to express the summer heat budget as g-cal per surface area ($g\text{-}cal/cm^2$).

7) To calculate the seasonal mean lake temperature, planimeter the area beneath each depth contour over the entire growing season, and determine the average temperature per depth interval (Steps 3-5). Calculate the weighted mean using interval volumes, and add 4 C.

Example

Our planimeter is calibrated as 90 degree-days per 146 units or 0.6164 degree-days per unit.

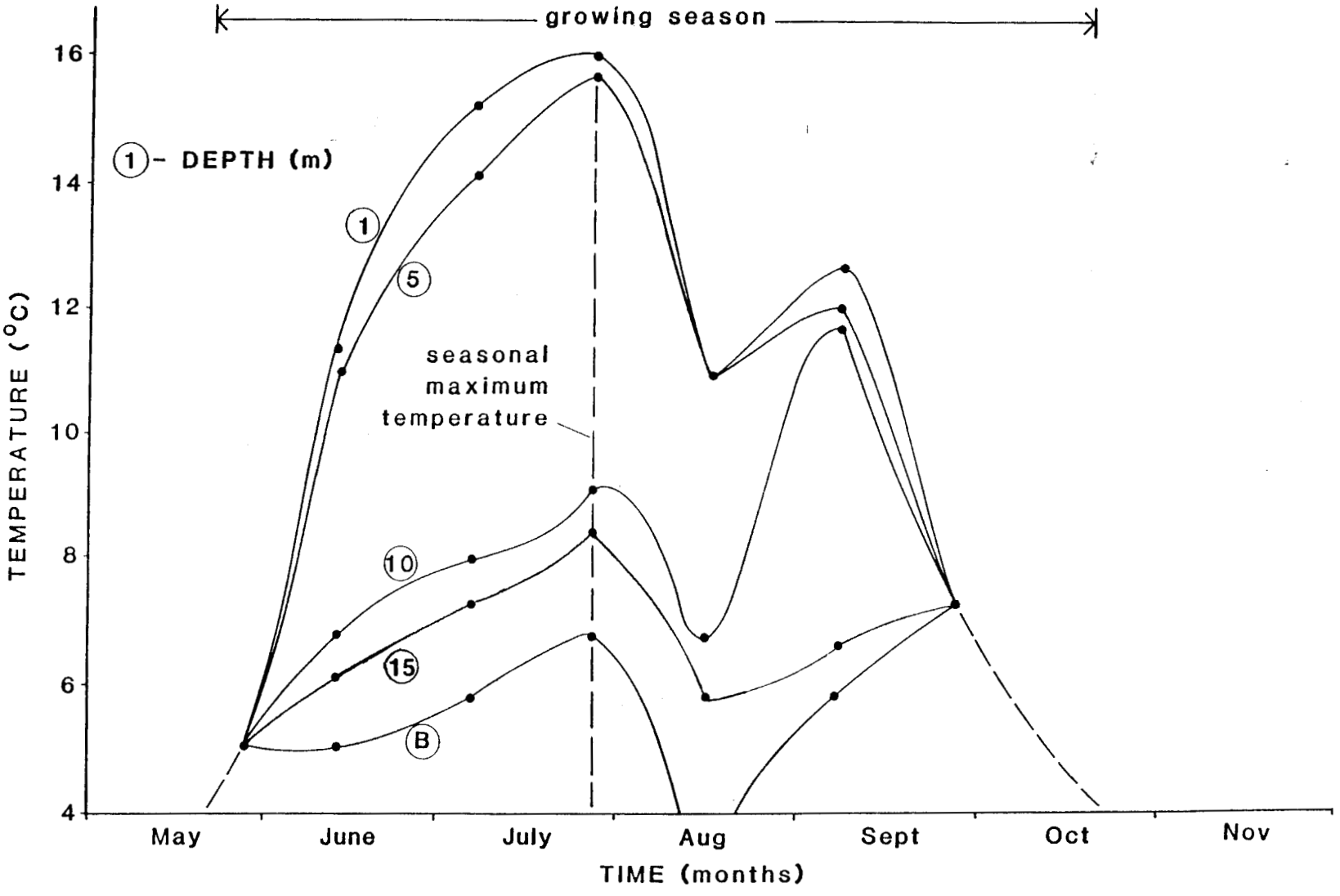


Figure 5. Isopleths of changing temperatures within strata made from field measures of vertical temperature profiles on seven dates over the growing season (isothermy in the spring to fall) used to determine the summer heat budget (4 C in the spring to the seasonal maximum temperature).

Table 1. Use of a planimeter to (A) estimate the average temperature of five depth increments; and (B) the calculation of accumulated gram-calories (g-cal) for each depth interval.

-A-

Depth increment (m)	Planimeter units (P)	Degree-days (P x 0.6164) (A)	Time to maximum temperature (d) (B)	Average temperature (A/B)
1	834	513	74	6.9
5	681	420	64	6.6
10	177	109	64	1.7
15	60	37	44	0.8
Lake bottom	35	22	44	0.5

-B-

Depth interval (m)	Average temperature (C)	Interval volume (ml) (D)	Gram-calories (C x D)
0-5	6.9	7.45×10^{12}	51.4×10^{12}
5-10	6.6	5.48×10^{12}	36.2×10^{12}
10-15	1.7	3.95×10^{12}	6.7×10^{12}
15-bottom	0.8	0.72×10^{12}	0.6×10^{12}

Total g-cal = 94.9×10^{12}

The surface area of the lake equals 1.8×10^{6} m^2 or 1.8×10^{10} cm^2. Therefore, the summer annual heat budget is:

$$\frac{94.9 \times 10^{12} \text{ g-cal}}{1.8 \times 10^{10} \text{ cm}^2} = 5{,}772 \text{ g-cal/cm}^2.$$

Dissolved Oxygen

One of the most significant gases found in lakes is oxygen. Dissolved oxygen (D.O.) is not only essential for aerobic respiration, but it influences all biochemical activities within an aquatic community. The distribution of D.O. is affected by temperature, photosynthesis, decomposition, and inorganic-chemical reactions. Oxygen solubility is inversely affected by temperature; i.e., D.O. levels increase with a decrease in temperature. Dissolved oxygen is measured using a combination D.O./temperature meter.

Procedure

Calibrate the meter (see Instrument Calibrations, p. 192), lower the probe while agitating if the probe is not equipped with an automatic stirrer, and record D.O. concentrations (mg L^{-1}) and temperatures.

Calculations

Measurements of D.O. are often converted to percent oxygen saturation using a solubility table, or more conveniently, by using a nomograph (Figure 6). Place a straight edge on the nomograph, and match the D.O. (mg L^{-1}) concentration with the corresponding temperature. Read the percent saturation directly from the graph.

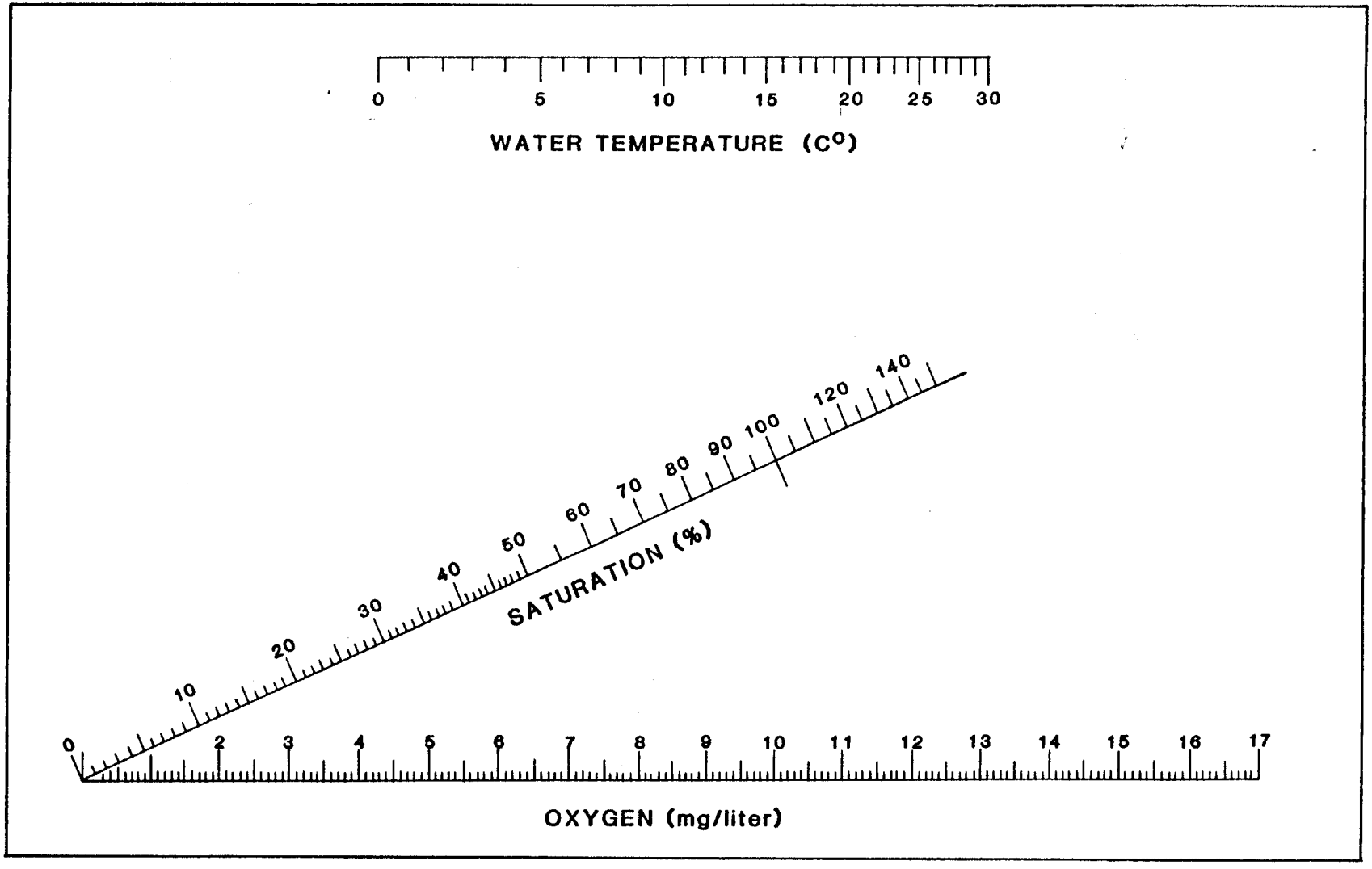


Figure 6. Nomograph for determining percent oxygen saturation using field measurements of dissolved oxygen ($mg \cdot L^{-1}$) and temperature (°C).

Photosynthetically Active Radiation (PAR)

Virtually all of the energy used in the metabolic processes within lake ecosystems is derived from sunlight. Solar radiation is converted to chemical energy by algal, bacterial and macrophytic photosynthesis. In addition, water absorbs light energy converting it to heat, thereby regulating the thermal structure of a lake. Measurements of light penetration are used to determine the 1% light level i.e., photosynthetic compensation point (CP) or the euphotic zone depth (EZD). This point refers to the maximal depth at which gross photosynthesis exceeds respiration, and defines the depth of the trophogenic zone. The CP depth varies seasonally and is affected by such factors as precipitation, algal production, and silt input.

A. Underwater Photometer

Procedure

Using an underwater photometer (sensitive to wavelengths of from 400-700 nm), record the incident light level by holding the probe just above the lake surface. Next, place the probe just below the surface (~5 cm), record the subsurface light level, and continue taking measurements at 0.5-meter intervals to a depth of 5 meters. Take additional readings at 1-meter intervals (if possible) until reaching ~1% of the subsurface light reading.

Calculations

1) Light-compensation point - Formulate a linear equation (Table 2) by regressing depth (Y-axis) against log (or ln) percent subsurface light (X-axis), and calculate the coefficient of determination (r^2). The Y-intercept of either regression formula gives the CP depth. Alternately, convert measurements to percent subsurface light, and plot on semi-log paper, the percent subsurface light (Y-axis) versus depth (X-axis). The X-intercept is the CP depth.

2) Euphotic volume - The penetration of 1% of the subsurface PAR delineates the deepest extent of the trophogenic zone and defines the euphotic zone depth (EZD). Multiply the EZD by lake surface area to estimate euphotic volume (trophogenic volume). Finally, the EZD is also used to define the extent of the littoral zone in lakes.

3) Light-extinction coefficient - The inverse slope of the ln regression line (K_d) is the light extinction coefficient

(m^{-1}) which can be obtained from conversion of the log slope to ln values e.g., log slope/2.3 equals ln slope.

Table 2. Light levels found at specific depths and use in calculating the light-compensation point (CP) and the light-extinction coefficient (K_d).

Depth (Y)	Light (footcandles)	Percent subsurface light (X)	Log (X)	ln (X)
Incident	3,700	--	--	--
Subsurface	3,500	100.0	2.00	4.605
0.5	2,800	80.0	1.90	4.382
1.0	2,600	74.3	1.87	4.308
4.0	2,100	60.0	1.78	4.094
6.5	1,400	40.0	1.60	3.689
7.5	950	27.2	1.43	3.303
10.0	655	13.7	1.14	2.617
15.0	295	8.4	0.92	2.128
22.0	145	4.1	0.61	1.411
30.0	30	0.9	-0.05	-0.105

Depth (m) = 29.2983 - 14.8851 (log % light)

$r^2 = .9861$

Depth (m) = 29.3535 - 6.4746 (ln % light)

$r^2 = .9859$

B. Secchi disk

The Secchi disk is a black and white circular plate, 20 cm in diameter, used to estimate the turbidity or degree of visibility in natural waters. The Secchi disk provides a very simple means of making transparency determinations in natural waters. A measured line is attached to the center of the disk by means of a special fitting that stabilizes the disk so that it will be parallel to the surface. The disk is lowered into the water until it just disappears from sight. The disk is raised and lowered and readings are taken from the calibrated line at the point where it disappears and reappears. The average of the two readings is recorded as the Secchi disk transparency (SD). Best results are obtained in the lee and/or shaded side of a boat, and are usually obtained after early morning and before late afternoon.

Transparency usually decreases in the summer when plankton, silt, and organic matter are more likely to be prevalent. The most transparent lakes are usually seepage lakes, because this characteristic greatly reduces the amount of silt-bearing influents. Drainage lakes carry more silt and usually are less transparent. For example, a drainage lake has a SD of 1.0 to 1.4 m, but a seepage lake in the same area may give readings of 3.0 to 4.0 m. A high reading of 19 to 21 m would indicate extreme clarity; however, that same lake in the summer may read only 10 m.

The SD is often used to estimate the euphotic zone depth (EZD) or light-compensation point within lakes. However, SD generally represents only a portion of the EZD which is rigorously defined through the use of a submersible photometer. The proportion of the EZD measured by SD varies by lake type and should be validated in each lake. However, we have developed regression equations that allow the EZD to be estimated, by lake type, from SD readings (Figure 7).

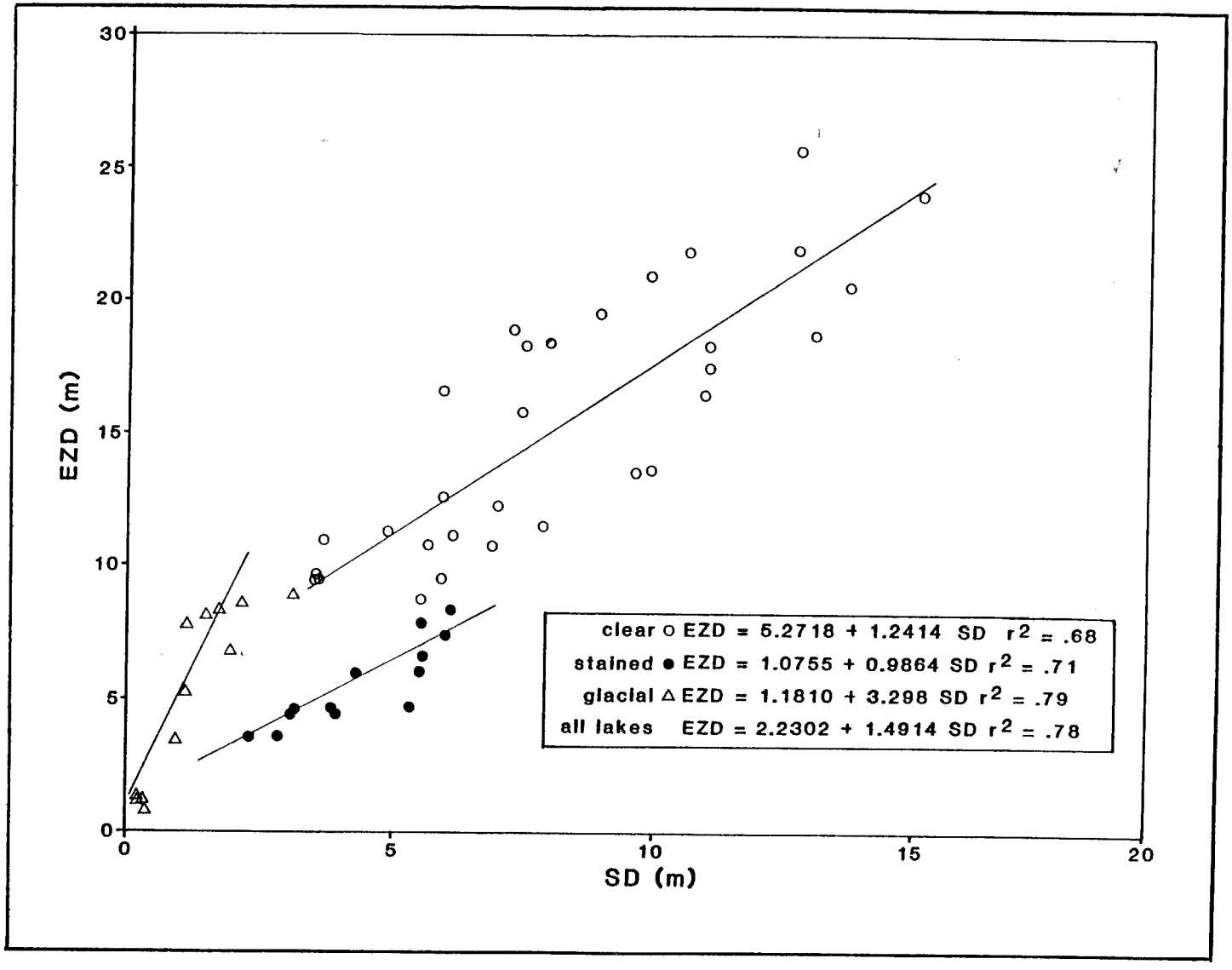


Figure 7. The relationship between the euphotic zone depth (EZD) defined by the 1% light level (determined by underwater photometer) and the Secchi disk transparency (SD) for glacial, organically stained, and clear-water lakes.

Stream Discharge

Stream discharge is the volume of water passing a point during a given period of time, it is related to flow rates and stream-bed characteristics. Mean flow rates are used to determine discharge and, when applied to an outlet stream, annual discharge rates are used to estimate the flushing rate or hydraulic retention time for lakes.

Procedure

1) Locate a fairly straight section of stream having a hard, uniform bottom. This gives a symmetrical pattern of flow within the stream.

2) Place a line or measuring tape across the stream to locate the cross-section at which the stream flow or velocity is to be determined.

3) With a current meter, measure the stream velocity at 60% of the depth from the surface or at 40% of the depth from the bottom (Figure 8) at selected intervals; i.e., divide the stream into equal segments (Figure 9).

4) Using the current meter instructions, determine the velocity (m/sec).

5) From the depth and distance, calculate the surface area of each cross-sectional portion of the stream (m^2) (Figure 9).

6) Average the stream velocity taken at the two sides of each section, and calculate the average discharge (m^3/sec).

7) To determine total stream discharge, sum the discharges for each section.

Example

Flow of section C is to be measured at 0.6 x 1 m, or 60 cm from the surface (40 cm from the bottom) and at 0.6 x 80 cm, or 48 cm from the surface (32 cm from the bottom) (Figure 9). If the velocity measurements equalled 3 m/sec and 3.5 m/sec, respectively, the discharge through Section C is calculated by:

$$\frac{[1\text{ m} + 0.8\text{ m}]}{2} \times 5\text{ m} \times \frac{[3\text{ m/sec} + 3.5\text{ m/sec}]}{2} = 14.63\ \text{m}^3/\text{sec}$$

The sum of discharges through sections A to J equals the total discharge of the stream.

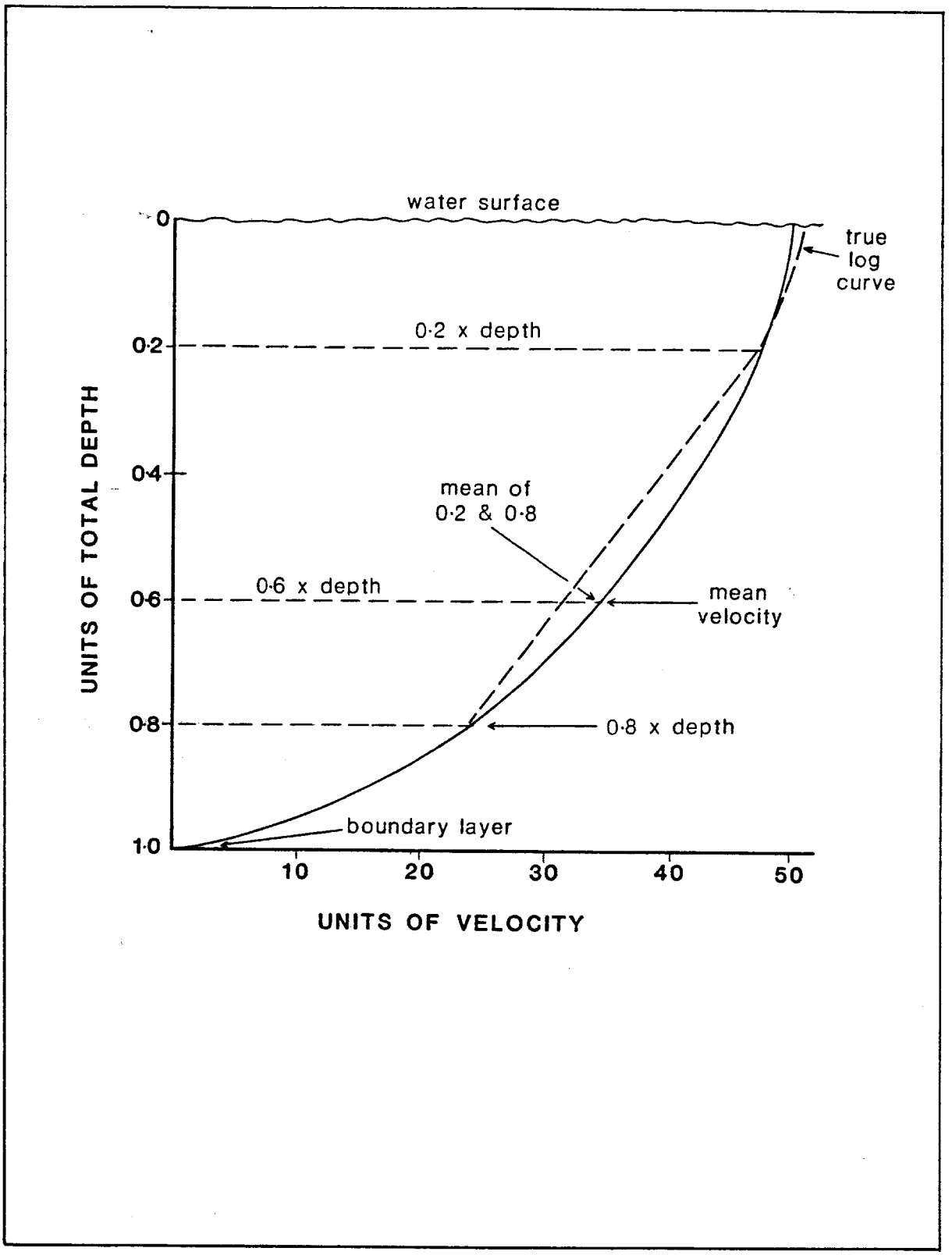


Figure 8. The relationship between depth and water velocity in an open channel showing the depths at which velocity can be measured to obtain a mean flow rate.

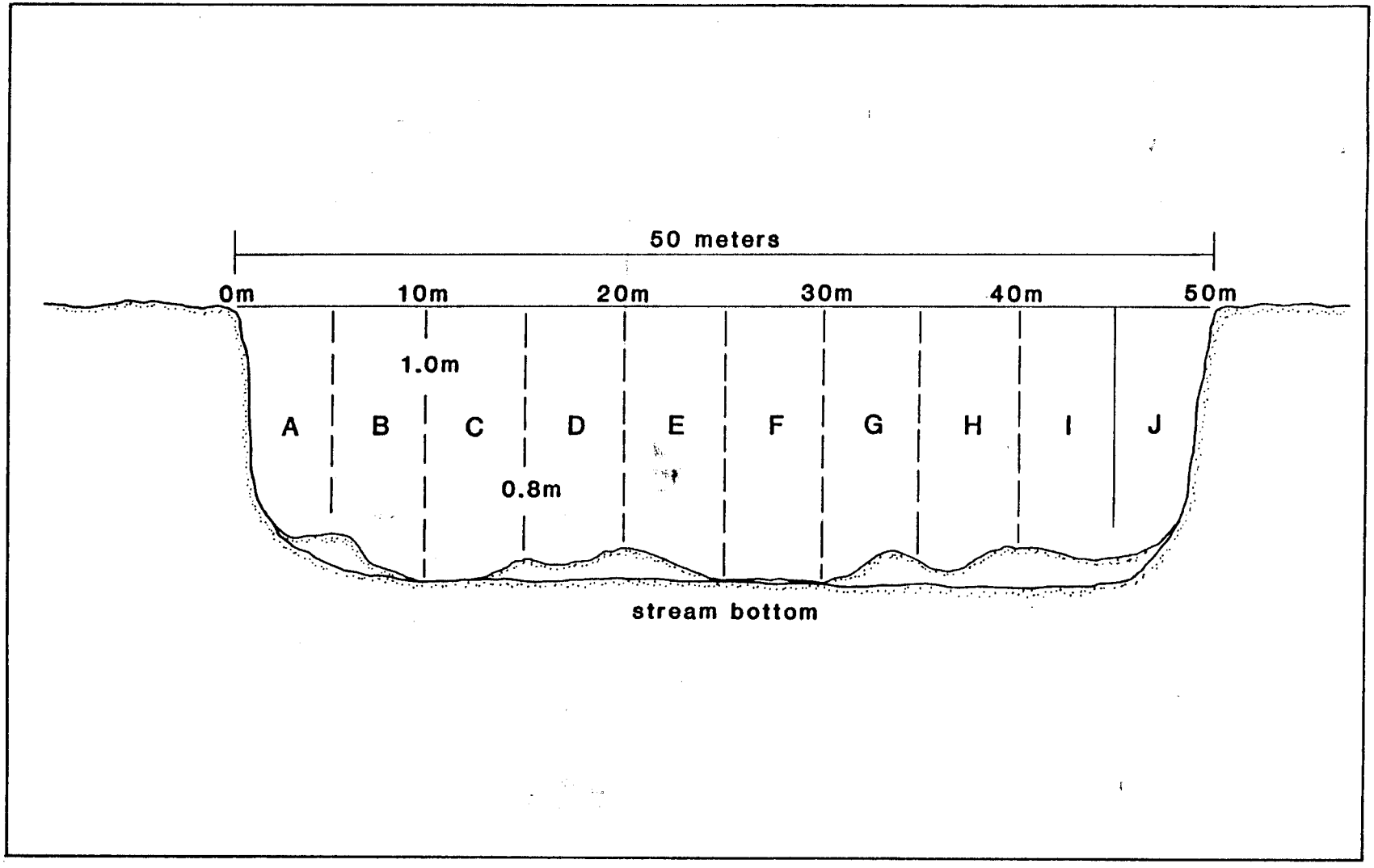


Figure 9. Stream cross section partitioned into segments and the segment boundaries at which water depths and flow rates are measured.

A. Staff Gauge Calibration

Discharge (Q) measurements are time consuming and often require several people to complete. Calibrated staff gauges offer a viable alternative to frequent discharge measurements. Briefly, a staff gauge is a strong rod, ruled in meters and centimeters (feet-inches) which is permanently anchored into the stream bottom. The anchorage is located in a quiet, but flowing area allowing only water level to affect the metered reading. Calibration is achieved by determining stream discharge and, at the same time, noting the water height on the ruled gauge. Readings are taken at both high and low water levels to provide for a broad range in discharges (Rantz et al. 1982).

Calculations

1) Formulate a linear equation by regressing discharges (Y-axis) against staff gauge readings (X-axis), and calculate the coefficient of determination (r^2).

2) To determine stream discharges, substitute staff gauge readings into the regression equation.

Example

Discharges were measured and the water height recorded for a staff gauge at the outlet of Falls Lake, Baranof Island, during 1981 and 1985. The log of 20 measured discharges (Y-axis) were regressed against staff gauge readings (X-axis) resulting in the equation:

$$\text{Log } Q = -0.537 + 0.0339 \text{ (cm)}; \; r^2 = 0.89$$

Using this equation, a 21.00 cm reading from the staff gauge would result in an estimated discharge of 1.50 m^3/sec.

B. Estimation of the Hydraulic (Water) Retention Time

The hydraulic (water) retention or water residence time is defined as the time required for the total volume of water within a lake to be replaced.

The method utilizes multiple regression analysis of lake and watershed characteristics versus known system discharges. The analysis has been carried out by the U. S. Forest Service and is delineated in the U. S. Forest Service Water Atlas (Anonymous 1979).

Southeast

Mean Annual Flow: $Q = 0.0312P^{1.13}A^{1.03}$ ($r^2 = 0.993$)

Southcentral

Mean Annual Flow: $Q = 0.0283P^{1.16}A^{1.02}$ ($r^2 = 0.997$)

Q = mean annual flow (cubic feet per second)
P = mean annual precipitation (inches)
A = watershed area (square miles)

[$Q \times 8.92 \times 10^5$ = total lake outflow (TLO) (m^3/yr)].

The theoretical water residence time is calculated according to the following:

$$T_w = V/_{TLO}$$

T_w = theoretical water residence time (yr)
V^w = total lake volume ($10^6 m^3$)
TLO = total lake outflow (10^6 m^3/yr)

NOTE: This method can only be used for lakes in areas covered by the U. S. Forest Service Water Atlas (1979).

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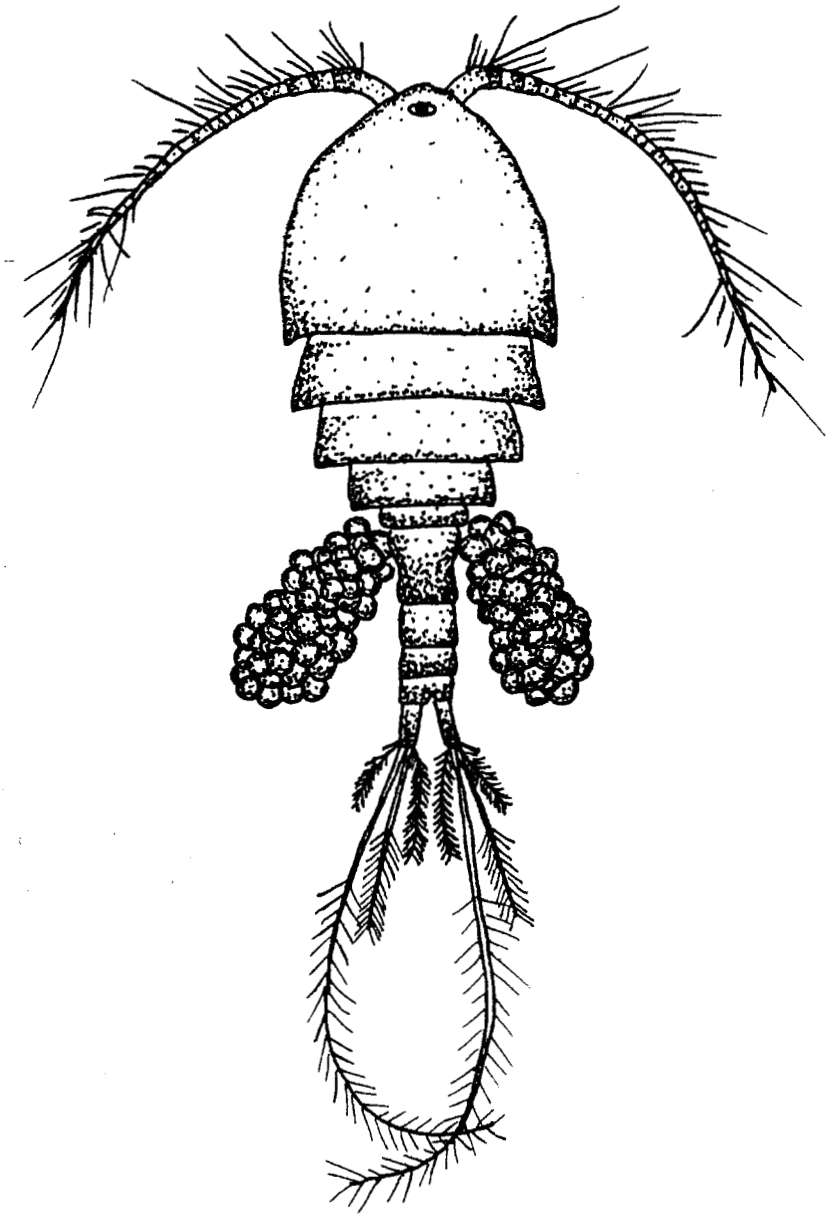
PART II.

LABORATORY TECHNIQUES AND METHODOLOGIES



SAFETY CONSIDERATIONS

The limnology staff recognizes the importance of laboratory safety and has developed an overall safety program. This has been accomplished and coordinated through an appointed safety officer whose primary responsibilities are (1) to recognize and eliminate both existing and potential health and safety hazards; and (2) train personnel to become aware of health and safety hazards. Laboratory personnel are informed of the State of Alaska's 'right to know' law that states 'all hazards of toxic and hazardous substances are evaluated and this information is to be transmitted to personnel at any time'. In compliance with this provision, the laboratory maintains material safety data sheets (MSDS) for all hazardous and toxic chemicals, and these are made available for inspection at any time. More importantly, laboratory personnel are trained to recognize safety hazards, and are instructed in the use of safety equipment and procedures designed to minimize or eliminate potential problems.

In addition, the limnology laboratory is equipped with basic first-aid supplies, fire extinguishers, emergency eye-wash and shower facilities. At least one person is trained in cardiac pulmonary resuscitation (CPR), and emergency telephone numbers (hospital, fire, poison-control center) are posted throughout the lab. Although it is beyond the scope of this manual to provide a complete laboratory health and safety reference, some basic procedures are listed here regarding general laboratory practices and for handling radioisotopes.

General Procedures

1) Never remove labels from any chemical-substance container.

2) Store chemicals according to the instructions listed on the manufacturer's label. Occupational Safety and Health Administration (OSHA) approved storage cabinets are to be used for flammables and corrosives. Bulk quantities of other chemicals are stored in a ventilated and locked storage bin.

3) Reagents, solutions, and chemicals routinely used for analysis are kept on shelves below waist level.

4) Smoking is prohibited in the laboratory at all times.

5) Wear protective clothing (e.g., laboratory aprons or coats, vinyl gloves, and protective eyewear) when handling hazardous or toxic chemicals.

6) Be especially careful when preparing alcoholic-phenol or dilute acids; protective clothing described above is mandatory.

7) Prepare volatile solutions (e.g., acetone) and chemicals only under the fume hood.

8) Always add concentrated acids or bases to water while using the fume hood.

9) Wipe up chemical spills immediately using the appropriate spill-control material.

10) Only autopipets are to be used for the transfer of solutions.

11) Be aware of laboratory safety and health hazards. Let your supervisors or safety officer know immediately if you have any questions.

Handling Byproduct Materials

1) While conducting experiments that utilize byproduct materials (carbon-14 and/or phosphorus-32), you will be required to use protective gear so marked; i.e., a laboratory coat/apron plus vinyl gloves. In addition, any transfer of the material in liquid form will require the use of automatic pipets and/or syringes. In essence, use prudent judgement in avoiding the possibility of direct contact of the material with your person.

2) We are required to monitor the byproduct use area after each experiment. The GM survey meter (model #493 and serial #1713, with probe 489-35) will be calibrated yearly by:

Victoreen Instrument, Inc.
NCR #34-0486-04
Repair and Calibration Dept.
10101 Woodland Avenue
Cleveland, Ohio 44104
(216) 795-8200

Records of the monitoring program are available from the limnology laboratory manager and are physically located within the limnology laboratory data files, Alaska Department of Fish and Game, Kalifornsky Road, Soldotna.

3) Use of byproduct material will be confined to the laboratory's sample preparation room using glassware so designated. Byproduct materials will be stored in a lockable refrigerator marked as containing radioactive materials. Use and storage areas are both located within

the sample-preparation room and are marked as containing radioactive materials (9" x 12" 'caution radioactive materials' signs on exterior doors). Signs conform to CFR 20 regulations of the Nuclear Regulatory Commission concerning color and appropriate warning.

4) You are required to follow good radiation safety practices which include but are not limited to the following:

   a) Use of byproduct material is restricted to the area so designated under Item 3 above.

   b) Smoking and the consumption of food and beverages is prohibited in the designated-use area.

   c) Contaminated areas will be cleaned using the decontaminants provided (e.g., 'Count-off' from New England Nuclear) with solid wastes being disposed of in the marked solid-waste containers. Potentially contaminated areas may be stained yellow to blue because of the addition of iodine to all liquid reaction vessels.

5) Each user will be issued personnel-monitoring devices consisting of thermoluminesent wrist dosimeters. Records of personnel-monitoring results (monthly) are kept in the office of the laboratory manager with the monitoring service provided by:

   Eberline
   P. O. Box 2108
   Santa Fe, NM 87501
   (505) 471-3232

6) Records of byproduct material use, transfer, and/or disposal will be kept and maintained within the laboratory. Each record will be updated, following an experiment, to include the location of the experiment, byproduct material used, quantity, method of disposal, and resultant total amount of byproduct remaining within the laboratory. Current byproduct-use records are located within the designated-use area, and all permanent records are located in the office of the laboratory safety officer.

7) Procedures for picking up, receiving, and opening packages containing byproduct materials are:

   a) Notify airline personnel that upon arrival of the material, limnology laboratory personnel are to be called at 262-5042 or 262-9368 from 8:00 a.m. to 4:30 p.m. or 262-5323 either before or after these hours.

b) Upon receiving a call that materials have arrived, pick up the container(s) within one working day.

c) As each package will contain no more than 10 mCi of carbon-14 or 5 mCi of phosphorus-32, the package exterior will not be monitored.

d) Transport the byproduct material to the laboratory by State of Alaska vehicle.

e) Shipping documents must accompany any and all transfer of radioactive materials on public roads.

f) Maintain current records of any transfer of radioactive materials either into or out of the laboratory.

g) Open packages only within the designated-use area, and inspect contents for leaks.

h) Open sealed containers containing byproduct material within the designated-use area. Record any broken ampules or glass vessels and clean or dispose contaminated surfaces as outlined below.

Byproduct Waste Disposal

1) Carbon-14 disposal will consist of:

a. Liquid waste (at $\leq$0.05 uCi per gm) will be returned to the lake system (remote site lake); i.e., 69-383 u Ci per lake per sampling period.

or

b. Liquid waste (at $\leq$0.05 uCi per gm) will be disposed of down a specified sink not to exceed 1 Ci per yr.

and

c. Solid wastes (e.g., vials and filters) and disposable items (e.g., gloves, absorbent materials, and pipet tips) will be buried at a sanitary-disposal site ($\leq$0.0225 u Ci per filter).

and

d. Liquid scintillation fluor (toluene based) will be allowed to evaporate from capped plastic vials placed outside in a secured area.

2) Phosphorus-32 disposal will consist of holding all liquid and solid wastes until decayed to background levels.

Emergency Procedures

These instructions are required reading for all personnel working within the limnology laboratory, regardless of whether you are or are not actively using byproduct materials.

1) Notify the lab manager upon the contamination of a work arena with carbon-14 in liquid form, and apply sodium bicarbonate to the spill. Clean the area with decontaminant scrubs located within the designated-use area.

2) Upon contaminating your person, wash the affected area with an alkaline detergent and copious amounts of water. Use decontaminant scrubs; e.g., Dri-Contrad, and notify the lab manager or safety officer.

3) Use survey instruments and wipe tests as described below to ensure that the area has been decontaminated:

   a) Monitor the of general area with a survey instrument sufficiently sensitive to detect at least 0.1 mR per hr carbon-14.

   b) Monitor bench tops, floors, etc., for contamination using 2.4-cm absorbent pads wiped over an area of 100 cm^2, and count using the Packard Liquid Scintillation spectrometer.

   c) As the designated-use area has its own water supply and disposal area as well as a separate entry-exit door, personnel pass-through will not be allowed until the area is cleared by the laboratory manager.

   d) Notify at least one of the following persons after initial containment of the spill:

J. P. Koenings
219 Crest Street
Soldotna, Alaska 99669
(907)262-5323 (home)
(907)262-9368

Jim Edmundson
P. O. Box 3155
Soldotna, Alaska 99669
(907)262-4172 (home)
(907)262-5042

Solutes, Solvents, and Solutions

Laboratory methodologies require preparing reagents, solutions, and standards from concentrated chemicals supplied either as liquids or as solids (crystals, powders, and pellets). In general, add the substance (solute) to an appropriate volumetric flask and slowly add the solvent, while mixing, until dissolved. Add additional solvent to reach the required volume. In addition, it is common laboratory practice to prepare a dilute (dil) solution from a concentrated (conc) stock solution. For example, the methodology may require 250 ml of 2 N sulfuric acid; however, the only acid available is 36 N. To calculate the required volume of 36 N acid use the expression:

$$C_{conc} \times V_{conc} = C_{dil} \times V_{dil}$$

The units of concentration (C) and volume (V) can vary, but must be the same on both sides of the expression. Using the above example, the calculation is:

$$36\ N \times V\ (ml) = 2\ N \times 250\ ml$$

$$V = 500/36 = 13.9\ ml$$

Therefore, dilute 13.9 ml of 36 N sulfuric acid to 250 ml with DI water to yield the required volume of a 2 N sulfuric acid solution.

Finally, samples may have concentrations exceeding the upper limit of detection of the methodology i.e., analytical results will be equivalent to the upper detection limit. Therefore, sample dilution is necessary prior to analysis to reduce concentrations to within operating limits. The correct concentration is then calculated by multiplying the concentration of the diluted sample by the dilution factor (DF) using:

$$DF = \frac{\text{volume of sample + volume of diluent}}{\text{volume of sample}}$$

For example, Fred Lake has a Kjeldahl nitrogen level of ~5000 ug L^{-1}, but the upper limit of detection for the method is 3000 ug L^{-1}. Diluting 5 ml of sample with 15 ml of water resulted in a concentration of 1,376 ug L^{-1}. The DF [(5 + 15)/5] equals 4, and the correct concentration of the original sample is 5,505 ug L^{-1} (1,376 x 4).

Conductivity

Specific conductance, conductivity, is defined as the measure of a solution's ability to carry an electric current. Resistance, the inverse of conductivity, is measured in ohms with conductivity being expressed as mhos/cm. In addition, conductivity can be expressed in Siemens (S), i.e., umhos/cm divided by 10 is equivalent to 1 mS/m. Measurements of conductivity are used to indicate levels of dissolved material and, specifically, as an indication of potential nutrient levels or fertility. As such, conductivity is often highly correlated with the amount of dissolved solids, and has been used in a procedure to determine salinity.

Accuracy

± 0.2% full scale at 10-200 umhos/cm.

Apparatus

YSI model-32 conductance meter, 100-ml graduate cylinder, 100-ml beakers, and a magnetic stirrer.

Reagents

Sodium chloride standard - A standard supplied by Hach Chemical Company has a conductivity of 1990 ± 10 umhos/cm at 25 C.

Procedure

1) Calibrate the meter according to the manufacturer's instructions. Check the accuracy of the meter by measuring the conductivity of the NaCl standard.

2) Pour 100 ml of sample into a beaker and place on a magnetic stirrer. Immerse the probe and allow the meter to reach equilibrium. Record the reading in umhos/cm.

3) Rinse the probe with DI water before continuing to the next sample.

4) Not all conductivity meters are temperature compensated. In addition, meters that are compensated for temperature vary in regard to compensation temperature. Thus, conversions are necessary to express conductivity at various standard temperatures; e.g., 18 C and 25 C. If the meter is not temperature compensated, use the following procedure to convert to a standard temperature:

    a) Determine the percent conductivity change per degree centigrade (% per C). Heat or cool a sample to exactly 25.0 C, and heat or cool a duplicate sample several degrees above or below 25.0 C.

b) Record the temperature and conductivities of the solutions, and calculate the slope from the expression:

$$\frac{\frac{C_{25} - C_x}{C_{25}}}{T_{25} - T_x} = \text{slope} = \% \text{ per } C$$

Where, C_{25} = conductivity of solution at 25 C.
C_x^{25} = conductivity of solution x at T_x.
T_{25}^x = 25 C.
T_x = temperature of solution x.

4) If the slope lies within the range of 0-4%, conductivity readings compensated to 25 C can be calculated from the expression,

$$\text{Conductivity (25 C)} = \frac{\text{sample conductivity}}{\text{slope}/4\% \times (0.04\text{-}1) + 1}$$

Salinity

Salinity can be determined from conductivity measurements. The ratio (R) of the sample conductivity at 15 C to that of a standard having a salinity of exactly 35 parts per thousand (%%) is used in a polynomial expression which defines salinity (Wooster et al. 1969). As conductivity increases with both increasing temperature and salinity; Table 3 provides a comparison of salinity, chlorinity, and conductivity at various temperatures.

Procedure

1) Prepare a salinity standard by dissolving 31.77 g of reagent grade NaCl in 1000 ml of DI water. This solution has a chlorinity of 19.4%% and a salinity of 35%%.

2) Measure the conductivity of the standard and the sample. Rinse the probe with DI water following the standard measurement before continuing to the sample. Solutions should be 15 C, and the meter's temperature compensator turned off.

Table 3. Laboratory results comparing salinity (‰), chlorinity (‰), and conductivity at various temperatures.

Salinity* (‰)	Chlorinity (‰)	Conductivity (umhos/cm) 5 C	15 C	20 C	25 C
0	0.0	0.48	0.86	1.19	1.34
5	2.8	5,090	6,180	7,120	7,670
10	5.5	9,610	12,150	13,600	15,600
15	8.3	14,080	17,340	19,500	21,500
20	11.1	17,960	20,100	25,100	28,000
25	13.8	22,000	26,200	30,200	33,800
35	19.4	30,300	36,500	40,300	44,700

*Salinity is determined from the expression S‰ = 1.80655 Cl‰ (after Wooster et al. 1969).

Calculation

$$R = \frac{\text{Conductivity of the sample}}{\text{Conductivity of the standard}}$$

Substitute R into the following polynomial expression, to define salinity in parts per thousand (‰):

$$\text{Salinity (‰)} = -0.09 + 28.30R + 12.81R^2 - 10.68R^3 + 5.99R^4 - 1.32R^5.$$

pH

pH units are a measure of the acidity of a solution at a specific temperature. Specifically, one unit of pH is equal to the negative log of the hydrogen ion concentration ($pH = -\log [H^+]$ where $[H^+]$ is the concentration of hydrogen ions in moles per liter).

pH is an important aspect of water quality, since almost every chemical reaction is pH dependent. Measurements of pH are also used in calculations of alkalinity and other acid-base equilibria.

Precision

± 0.02 pH (pH range); ± 0.01 pH (pH expanded range).

Accuracy

± 0.05 pH (pH range); ± 0.005 pH (pH expanded range).

Apparatus

Orion model-399A/ion analyzer, 100-ml graduate cylinder, 100-ml beakers, and magnetic stirrer.

Reagents

Buffer solutions of pH 4, 7, and 10.

Procedure

1) Calibrate the pH meter (see Instrument Calibrations, p. 192).

2) Allow the samples to reach room temperature (~25 C).

3) Pour 100 ml of sample into a beaker and place on a magnetic stirrer. Immerse the probe, allow the meter to reach equilibrium, and record the pH.

4) Rinse the probe with DI water before continuing to the next sample

Alkalinity

The alkalinity of lake water is largely due to the presence of carbonate ($CO_3^=$) and bicarbonate (HCO_3^-) ions. The proportion of each species is a function of pH. Since these ions are converted to carbon dioxide (CO_2) at pH 4.5, this procedure determines the total amount of inorganic carbon available for algal uptake (Table 40). Also, the amount of alkalinity determines the ability of natural waters to resist changes in pH. Such buffering capacity is important as many chemical reactions e.g., photosynthesis, affect the pH.

Limit of Detection

0.5 mg L^{-1} as $CaCO_3$.

Apparatus

pH meter, magnetic stirrier, 100-ml graduate cylinder, 100-ml beakers, and a 10-ml automatic buret (0.1 ml divisions).

Reagents

1) 1 N Sulfuric acid - Dilute 27.8 ml of concentrated H_2SO_4 to 1 liter with DI water.

2) 0.02 N Sulfuric acid standard - Dilute 10 ml of 1 N H_2SO_4 to 500 ml with DI water.

3) Standard pH 4 and 7 buffers.

Procedure

1) Calibrate the pH meter (see Instrument Calibrations, p. 192).

2) Pour 100 ml of sample into a beaker and place on a magnetic stirrer. Immerse the pH probe in the sample.

3) Using the buret slowly add titrant (0.02 N H_2SO_4) to a pH of 4.5. Record the volume (ml) of titrant.

4) Rinse the probe with DI water before continuing to the next sample.

Calculations

$$\text{Total alkalinity (mg } L^{-1} \text{ as } CaCO_3) = \frac{B \times N \times 50000}{V}$$

Where: B = ml titrant (0.02 N H_2SO_4)
N = normality of the titrant
V = sample volume (ml)

Turbidity

Turbidity is primarily caused by suspension of inorganic material (silt) and organic particles (algal cells) in the water column. Measurements of turbidity are made by comparing a sample's ability to scatter light to that of a known reference. The unit of measurement is the nephelometric turbidity unit (NTU). As suspended material acts to decrease the depth of the euphotic zone (EZD), measurements of turbidity are used to evaluate the photic qualities of lakes (Figure 10).

Apparatus

HF Instruments model-DRT 100 turbidimeter with reference standard, and a 30-ml cuvette.

Procedure

1) Calibrate the turbidimeter with a reference standard according to the manufacturer's instructions.

2) Invert an unfiltered sample several times and pour into a cuvette.

3) After all air bubbles have dissipated, record the NTU reading from the appropriate scale.

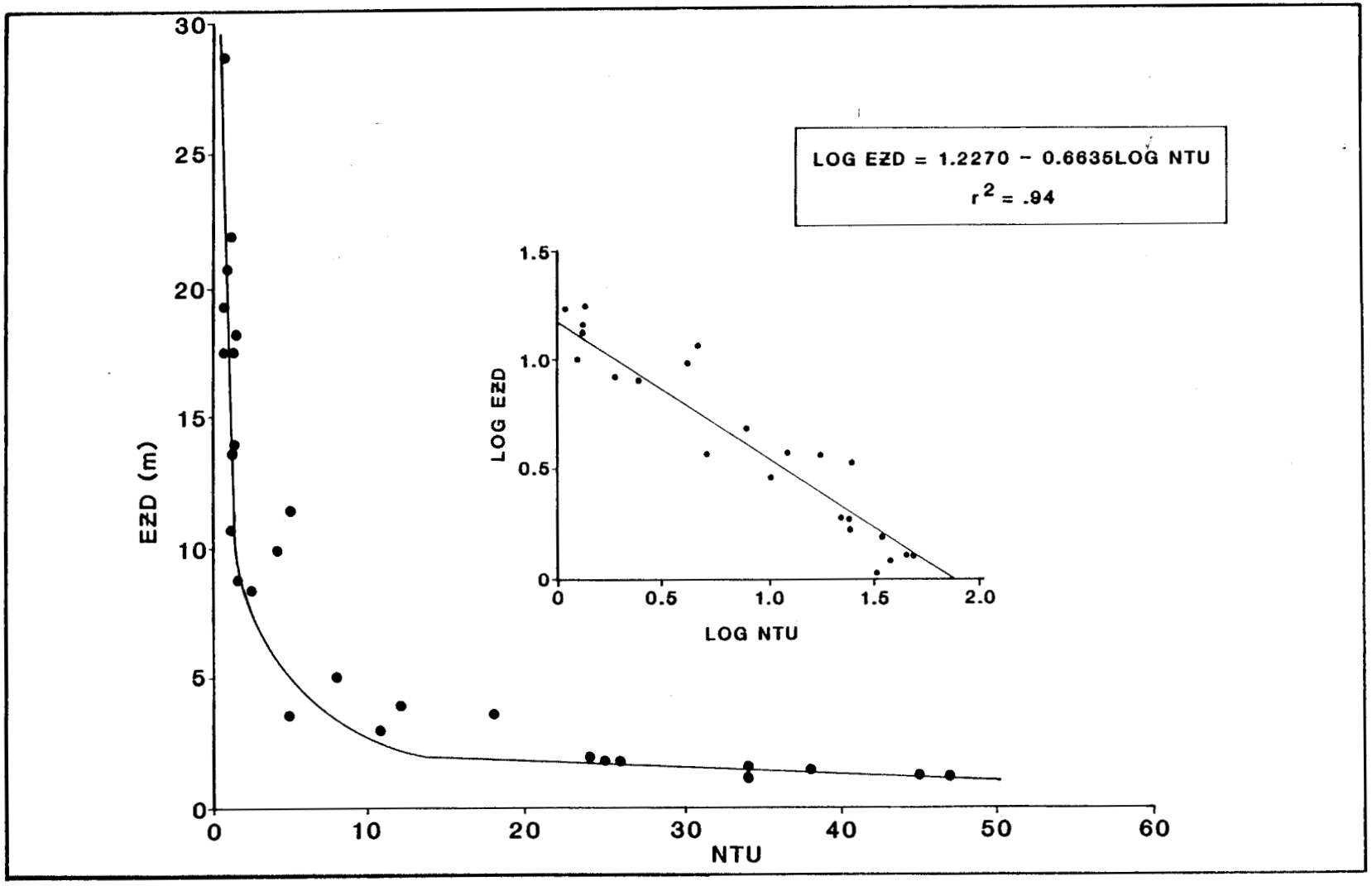


Figure 10. The relationship of decreasing euphotic zone depth (EZD) to increasing turbidity (NTU), and the log-log transformation and linear regression equation.

Color

The presence of organic compounds and colloidal particles impart true color to lake water, and both act to restrict light penetration (Figure 11). In contrast, apparent color is imparted to lakes by suspended inorganic material which also causes turbidity.

The color of filtered lake water is determined by comparing absorbance at 400 nm to a standard containing a known amount of platinum cobalt (Pt).

Apparatus

Spectrophotometer (400 nm) and 10-mm cuvettes.

Reagents

Color standard - Dissolve 1.246 g of potassium chloroplatinate (K_2PtCl_6) and 1 g of cobaltous chloride ($CoCl_2$ $6H_2O$) in 500 ml of DI water containing 100 ml of concentrated HCl and dilute the mixture to 1 liter with DI water. Alternately, a 500 platinum cobalt unit standard is supplied by Hach Chemical Company.

Procedure

1) Prepare a serial dilution of the color standard (Table 4).

2) Measure the absorbance (abs) of standards at 400 nm against a DI water blank.

3) Formulate a linear equation by regressing platinum cobalt units (Y-axis) against absorbances (X-axis) and calculate the coefficient of determination (r^2).

4) Measure the absorbance of a filtered sample at 400 nm against a DI water blank (see Instrument Calibrations, p. 192).

Calculations

Calculate sample color (Pt units), by substituting sample absorbances into the regression formula (Table 4).

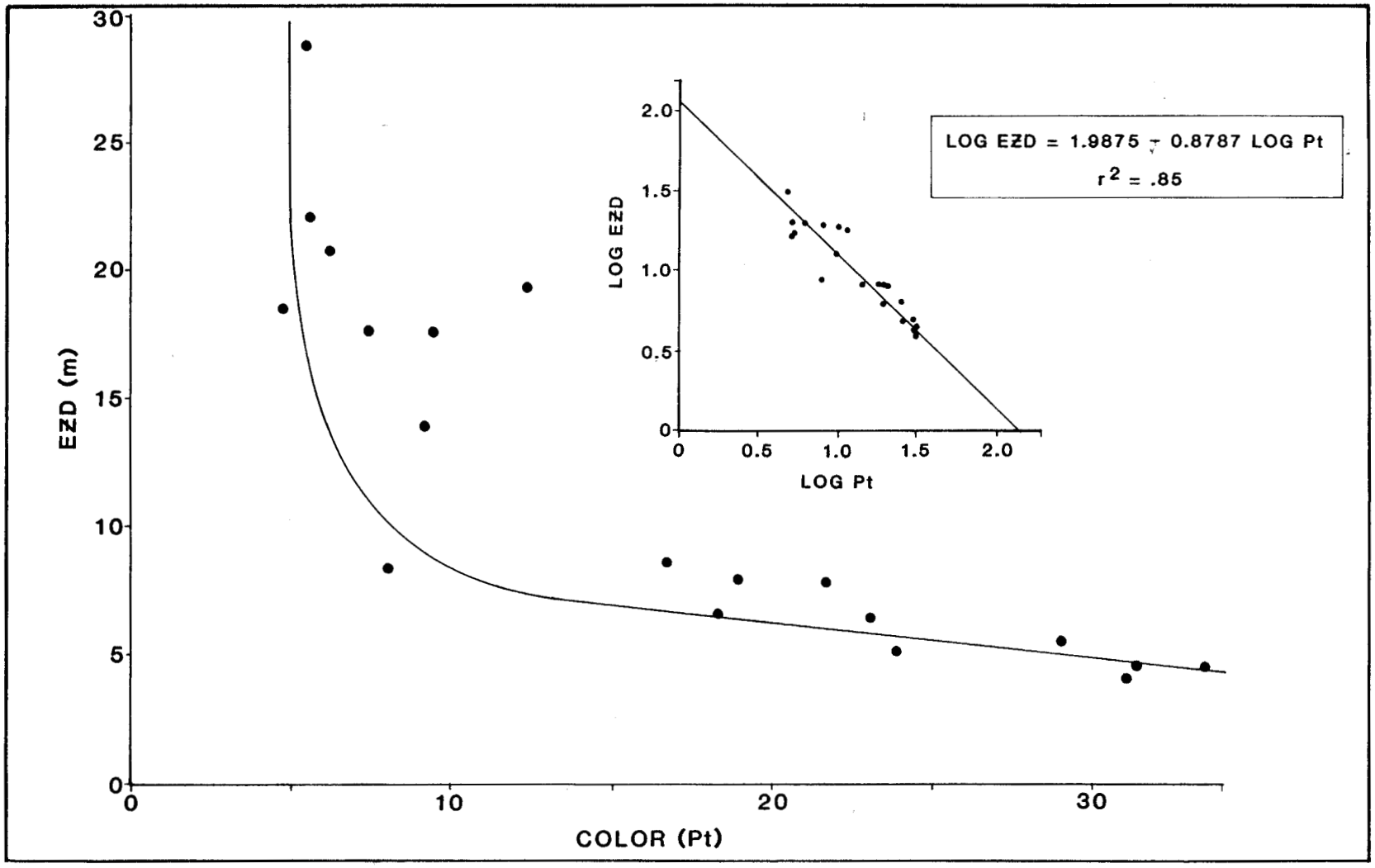


Figure 11. The relationship of decreasing euphotic zone depth (EZD) to increasing lake-water color, expressed as platinum cobalt units (Pt), and the log-log transformation and linear regression formula.

Table 4. Representative values used to establish the relationship between absorbances (abs) at 400 nm and platinum-cobalt units (Pt).

Volume of color standard (ml)	Volume of DI (ml)	Platinum cobalt units (Pt)	Absorbance (400 nm)
4.0	0.0	500.0	.443
3.9	0.1	487.5	.431
3.8	0.2	475.0	.420
3.7	0.3	462.0	.408
3.5	0.5	437.5	.386
3.0	1.0	375.0	.332
2.0	2.0	250.0	.219
1.0	3.0	125.0	.108
0.5	3.5	62.5	.054
0.3	3.7	37.5	.033
0.2	3.8	25.0	.019
0.1	3.9	12.5	.009
0.0	4.0	0.0	.000

Pt units = 1.9104 + 1126.8922 (abs)

$r^2 = 1.0000$

Total, Suspended, and Dissolved Solids

Total dissolved solids (TDS) is the residue formed upon evaporation of filtered water, and total solids (TS) the residue formed after evaporation of unfiltered water. As the TDS determination includes dissolved (ions) as well as suspended material, values can be correlated to both conductivity and alkalinity. TS values can include sizeable amounts of suspended solids (TSS) which impart turbidity.

Limit of Detection

2 mg L^{-1} residue.

Apparatus

Electronic balance with minimal sensitivity of 0.0001 g, drying oven, hot plate, and 30-ml petri dishes.

Procedure

1) Place a clean petri dish in the drying oven at 100 C for 4 hours.

2) Desiccate for at least 4 hours, and weigh to the nearest 0.0001 g (W_o).

3) Pour 30 ml (0.03 l) of filtered (TDS) or unfiltered (TS) sample into the petri dish, and evaporate the water at 80 C on a hot plate. Evaporate an additional 30-ml aliquot for a total sample volume of 0.06 l.

4) Place in the drying oven at 100 C for an additional 4 hours.

5) Desiccate the sample for 4 hours, and weigh (W_f).

Calculations

TDS and TS are calculated using the following expression:

$$\text{TDS or TS (mg L}^{-1}) = \frac{(W_f - W_o)}{0.060\ l} \times 1000\ \text{mg/g}$$

NOTE: Suspended solids (TSS) can be determined by difference using the expression TS = TDS + TSS.

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Calcium and Magnesium (titration)

Calcium (Ca) and magnesium (Mg) are two of the more important elements contributing to water hardness. Both elements can be determined in the same sample by first precipitating out Mg; and then determining Ca. Ca forms an orange compound with glyoxal, but forms a stronger bond with EDTA. As EDTA is added, the color changes from orange to yellow. Addition of acid dissolves the Mg precipitate and destroys the Ca-EDTA complex, allowing for a separate titrametric determination of Mg.

Standard Range

2.5 - 12.5 mg L^{-1} Ca, 1.5 - 7.5 mg L^{-1} Mg.

Upper Limit of Detection

150 mg L^{-1} Ca, 175 mg L^{-1} Mg.

Lower Limit of Detection

Empirical: 0.3 mg L^{-1} Ca, 0.3 mg L^{-1} Mg.

Predicted: 0.2 mg L^{-1} Ca, 0.2 mg L^{-1} Mg.

Precision

11% at 5 mg L^{-1} Ca, 21% at 3 mg L^{-1} Mg.

Accuracy

$\pm$ 6% at 5 mg L^{-1} Ca; $\pm$ 11% at 3 mg L^{-1} Mg.

Apparatus

pH meter, magnetic stirrer, graduate cylinder, 100-ml beakers, and 10-ml automatic buret (0.1-ml divisions).

Reagents

1) 0.01 N EDTA - Dissolve 1.861 g EDTA-disodium salt into ~400 ml of DI water, and dilute to 500 ml.

2) Glyoxal indicator - Dissolve 0.3 g glyoxal bis-(2-hydroxyanil) into 100 ml of reagent-grade methanol. Warm gently to dissolve.

3) 0.1 N NaOH - Dissolve 4 g of NaOH into ~500 ml of DI water, and dilute to 1 liter.

4) 1 N HCl - Dilute 21.0 ml of concentrated HCl to 250 ml with DI water.

5) Borax buffer - Dissolve 10 g of sodium borate - 10 hydrate in ~200 ml of DI water. Add 2.5 g of NaOH, and 1.25 g of sodium sulfide-9-hydrate previously dissolved in 25 ml of DI water. Combine the two solutions, and dilute the mixture to 250 ml with DI water.

6) Eriochrome Blue-Black B (C.I. 14640) indicator - Mix together 0.4 g of Eriochrome Blue-Black B and 100 g of sodium chloride.

Standards

1) Ca standard (1 mg ml^{-1} Ca) - Dissolve 1.838 g of calcium chloride-dihydrate into ~450 ml of DI water, and dilute to 500 ml.

2) Mg standard (0.6 mg ml^{-1} Mg) - Dissolve 2.510 g magnesium chloride-6-hydrate into ~450 ml of DI water, and dilute to 500 ml.

Procedure

1) Prepare Ca and Mg standards in the same beakers according to Tables 5 and 6.

2) Calibrate the pH meter using pH 4 and 10 buffers (see Instrument Calibrations, p. 192).

3) Pour 40 ml of sample or combined standards into a beaker and place on a magnetic stirrer.

A. Calcium

4) Add 5.0 ml of 0.1 N NaOH.

5) Add 3.0 ml of glyoxal indicator.

6) Allow 1-3 minutes for color development (orange-red).

7) Using the automatic buret, titrate the solution with 0.01 N EDTA until the color change of orange-red to yellow.

8) Record the volume (ml) of titrant and re-zero the buret.

B. Magnesium

9) Add a varying amount (~0.5 ml) of 1 N HCl using an autopipet to pH 4.0.

10) Add 1 ml of borax buffer (pH should equal 10.1), and ~100 mg of Eriochrome Blue-Black indicator.

11) Titrate the solution with 0.01 N EDTA until the color change of violet to blue, and record the volume (ml) of titrant.

Calculations

1) Formulate separate linear equations for calcium and magnesium by regressing concentrations (Y-axis) against volumes (ml) of EDTA (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by substituting volumes (ml) of EDTA into the appropriate regression formula (Tables 5 and 6).

Table 5. The relationship between known concentrations of calcium and the volumes (ml) of ETDA titrant used to calculate a standard curve in the analysis of calcium.

Calcium standard (ml)	Total volume (L)	Calcium concentration (mg L^{-1})	EDTA titrant (ml)
0.0	.040	0.0	0.0
0.1	.040	2.5	0.3
0.2	.040	5.0	0.6
0.3	.040	7.5	0.9
0.5	.040	12.5	1.4

$$Ca\ (mg\ L^{-1}) = -0.1792 + 8.8737\ (EDTA)$$
$$r^2 = .9977$$

Table 6. The relationship between known concentrations of magnesium and volumes (ml) of EDTA titrant used to calculate a standard curve in the analysis of magnesium.

Magnesium standard (ml)	Total volume (L)	Magnesium concentration (mg L^{-1})	EDTA titrant (ml)
0.0	.040	0.0	0.0
0.1	.040	1.5	0.4
0.2	.040	3.0	0.6
0.3	.040	4.5	1.0
0.5	.040	7.5	1.6

$$Mg\ (mg\ L^{-1}) = -0.0968 + 4.7177\ (EDTA)$$
$$r^2 = .9964$$

Calcium (colorimetric)

This method is adapted from Golterman (1970). The principle involves the reaction of calcium with glyoxal to form an orange-red compound. The color remains linear with calcium concentration over a relatively small range; however, this procedure is quite applicable for calcium levels not exceeding ~5 mg L^{-1}.

Standard Range

0.2-4.0 mg L^{-1}.

Upper Limit of Detection

5 mg L^{-1} Ca.

Lower Limit of Detection

Empirical: 0.10 mg L^{-1} Ca.
Predicted: 0.04 mg L^{-1} Ca.

Precision

3% at 1.2 mg L^{-1} Ca.

Accuracy

$\pm$ 5% at 1.2 mg L^{-1} Ca.

Apparatus

Spectrophotometer (520 nm), 15-ml disposable centrifuge tubes, and 10-mm cuvettes.

Reagents

1) Buffer - Dissolve 5 g of sodium hydroxide and 5 g of sodium borate-10-hydrate into ~450 ml of DI water, and dilute to 500 ml.

2) Glyoxal indicator - Dissolve 0.5 g of glyoxal bis-(2-hydroxyanil) into 100 ml of reagent-grade methanol.

3) Reagent alcohol, anhydrous.

Procedure

1) Pipet 5 ml of unfiltered sample or standard into a 15-ml disposable centrifuge tube.

2) Add 0.5 ml of buffer.

3) Add 0.25 ml of glyoxal indicator.

4) Add 5 ml of reagent alcohol and invert to mix.

5) Allow 25 minutes for color development, and measure the absorbance at 520 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary standard (0.2 mg ml^{-1} Ca) - Dissolve 0.3676 g of calcium chloride-dihydrate ($CaCl_2$-H_2O) into ~450 ml of DI water, and dilute to 500 ml.

Secondary standard (0.02 mg ml^{-1}) - Dilute 10 ml of the primary standard to 100 ml with DI water.

Calculations

1) Formulate a linear equation by regressing concentration (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 7).

Table 7. The relationship between known concentrations of calcium and absorbances ($\bar{abs}$) at 520 nm used to calculate a standard curve in the colorimetric analysis of calcium.

Secondary standard (ml)	Total volume (L)	Calcium concentration (mg L^{-1})	Absorbance (x)	$\bar{\bar{abs}}$ ($\bar{x}$-blank)
0.00	.005	0.0	.059, .057	.000
0.05	.005	0.2	.093, .094	.036
0.10	.005	0.4	.124, .128	.068
0.30	.005	1.2	.278, .288	.225
0.50	.005	2.0	.446, .451	.373
1.00	.005	4.0	.806, .809	.751

$$Ca\ (mg\ L^{-1}) = 0.0141 + 5.3101\ (\bar{\bar{abs}})$$
$$r^2 = .9999$$

Magnesium (colorimetric)

Magnesium reacts with Brilliant Yellow dye to form an orange-colored complex (Taras 1948). The color remains linear with magnesium concentration over a relatively small range; however, this procedure is applicable for magnesium levels below ~5 mg L^{-1}.

Standard Range

0.4-4.0 mg L^{-1} Mg.

Upper Limit of Detection

5 mg L^{-1} Mg.

Lower Limit of Detection

Empirical: 0.4 mg L^{-1} Mg.
Predicted: 0.24 mg L^{-1} Mg.

Precision

24% at 1.2 mg L^{-1} Mg.

Accuracy

$\pm$ 20% at 1.2 mg L^{-1} Mg.

Apparatus

Spectrophotometer (540 nm), 15-ml disposable centrifuge tubes, and 10-mm cuvettes.

Reagents

1) 1.0 N Sulfuric acid - Add 28 ml of concentrated H_2SO_4 to 500 ml of DI water, cool, and dilute to 1 liter.

2) 0.1 N Sulfuric acid - Dilute 50.0 ml of 1 N sulfuric acid to 500 ml with DI water.

3) 5 N Sodium hydroxide - Dissolve 100 g of sodium hydroxide into ~400 ml of DI water, cool, and dilute to 500 ml.

4) Calcium-aluminum solution - Dissolve 0.77 g of aluminum sulfate ($Al_2[SO_4]_3$) into ~500 ml of DI water. Add 62.9 g of calcium chloride-dihydrate, 40 ml of concentrated nitric (HNO_3)acid, and dilute to 1 liter with DI water.

5) 2% Starch solution - Add 10 g of soluble starch to 200-300 ml of DI water. Add 5 N sodium hydroxide while stirring until the solution becomes clear, and add 1 ml of glacial acetic acid. Dilute the mixture to 500 ml with DI water, and filter through a 'fast' pleated filter.

6) Brilliant Yellow (C.I. 24890) indicator - Dissolve 0.017 g of Brilliant Yellow Dye into ~450 ml DI water, and dilute to 500 ml. Prepare fresh daily.

Mixed Reagents

1) Mixed reagent I (MR I) - Combine equal volumes of 0.1 N sulfuric acid and calcium-aluminum solution.

2) Mixed reagent II (MR II) - combine equal volumes of 5 N NaOH and 2% starch solution.

Procedure

1) Place 5 ml of sample or standard in a 15-ml centrifuge tube.

2) Add 0.2 ml of MR I and invert to mix.

3) Add 1.0 ml of MR II and invert to mix.

4) Add 3.0 ml of the indicator solution and invert to mix.

5) Allow at least 20 minutes for full color development, and measure the absorbance at 540 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary standard (0.2 mg ml^{-1} Mg) - Dissolve 0.508 g of magnesium sulfate-heptahydrate ($MgSO_4$-7 H_2O) into 200 ml of DI water, and dilute to 250 ml.

Secondary standard (0.02 mg ml^{-1} Mg) - Dilute 10 ml of the primary magnesium standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank from the sample absorbances, and substitute into the regression formula (Table 8).

Table 8. The relationship between known concentrations of magnesium and absorbances ($\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}}$) at 540 nm used to calculate a standard curve in the colorimetric analysis of magnesium.

Secondary standard (ml)	Total volume (L)	Magnesium Concentration (mg L^{-1})	Absorbance (x)	$\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}}$ ($\bar{x}$-blank)
0.0	.005	0.0	.656, .686	.000
0.1	.005	0.4	.693, .698	.025
0.2	.005	0.8	.718, .743	.060
0.3	.005	1.2	.753, .751	.081
0.5	.005	2.0	.767, .764	.095
1.0	.005	4.0	.810, .810	.139

$$Mg\ (mg\ L^{-1}) = \bar{\bar{0}}.4248 + 27.3719\ (\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}})$$
$$r^2 = .8912$$

Total Iron (manual)

Iron (Fe) is an essential nutrient for phytoplankton, and chemically is closely coupled with the phosphorus cycle. In natural waters iron exists in two oxidation states ferric (Fe III) and ferrous (Fe II); and in particulate as well as in organic colloidal complexes. In natural waters, iron in chemical equilibrium is present at levels $\leq$20 ug L^{-1}.

Total iron is determined by reducing ferric iron to ferrous iron with hydroxylamine and hydrochloric acid during heat digestion. Ferrous iron reacts with Ferrozine to form a violet-red compound which is measured colorimetrically.

Standard Range

10-200 ug L^{-1} Fe.

Upper Limit of Detection

7000 ug L^{-1} Fe.

Lower Limit of Detection

Empirical: 4 ug L^{-1} Fe.
Predicted: 3.3 ug L^{-1} Fe.

Precision

17% at 40 ug L^{-1} Fe.

Accuracy

$\pm$ 7% at 40 ug L^{-1} Fe.

Apparatus

Spectrophotometer (562 nm), autoclave (121 C, 15 psi), 50-ml erlenmeyer flasks, and 10- or 40-mm cuvettes.

Reagents

1) Acid-reductant - Dissolve 10 g of hydroxylamine-hydrochloride into 100 ml of 1:1 hydrochloric (HCl) acid.

2) 6 N sodium hydroxide - Dissolve 120 g of NaOH into ~400 ml of DI water, cool, and dilute to 500 ml.

3) Sodium acetate solution - Dissolve 75 g of sodium acetate-trihydrate into ~400 ml of DI water. Warm the solution to dissolve, and dilute to 500 ml.

4) Ferrozine (iron reagent) - Commercially supplied by Hach Chemical Company, the reagent is pre-mixed and ready to use. Store in the dark at room temperature; however, a precipitate may occur after prolonged storage, so thoroughly mix prior to use.

Procedure

1) Pour 30 ml of unfiltered sample or standard into a 50-ml erlenmeyer flask.

2) Add 1 ml of acid-reductant and swirl to mix.

3) Cover the flask with a 15-ml polypropylene beaker and autoclave (121 C, 15 psi) for 15 minutes.

4) Cool to room temperature and add in order: 1 ml of Ferrozine, 2 ml of sodium acetate, and 2 ml of 6 N sodium hydroxide. Swirl to mix and allow 20 minutes for full color development.

5) Measure the absorbance at 562 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary standard (0.03 mg ml^{-1} Fe) - Dissolve 0.0726 g of ferric chloride-6-hydrate into ~400 ml of DI water containing 2.5 ml of 1:1 sulfuric acid, and dilute to 500 ml with DI water.

Secondary standard (0.3 ug ml^{-1} Fe) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against the averaged absorbances minus the blank ($\bar{X}$-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Tables 9 and 10).

Table 9. The relationship between known concentrations of iron and absorbances ($\bar{\bar{abs}}$) at 562 nm (10-mm cuvette) used to calculate a standard curve in the manual analysis of total iron.

Volume of secondary standard (ml)	Total volume (L)	Iron concentration (ug L^{-1})	Absorbance (x)	$\bar{\bar{abs}}$ ($\bar{x}$-blank)
0	.030	0	.011, .011	.000
1	.030	10	.015, .015	.004
4	.030	40	.027, .027	.016
10	.030	100	.052, .051	.041
14	.030	140	.067, .064	.055
20	.030	200	.089, .091	.079

$$Fe\ (ug\ L^{-1}) = -0.5903 + 2530.9843\ (\bar{\bar{abs}})$$
$$r^2 = .9996$$

Table 10. The relationship between known concentrations of iron and absorbances ($\bar{\bar{abs}}$) at 562 nm (40-mm cuvette) used to calculate a standard curve in the analysis of total iron.

Volume of standard (ml)	Total volume (L)	Iron concentration (ug L^{-1})	Absorbance (x)	$\bar{\bar{abs}}$ ($\bar{x}$-blank)
0	.030	0	.078, .078	.000
1	.030	10	.098, .096	.019
4	.030	40	.151, .156	.076
10	.030	100	.249, .249	.171
14	.030	140	.317, .315	.238
20	.030	200	.431, .419	.347

$$Fe\ (ug\ L^{-1}) = -1.1344 + 583.7913\ (\bar{\bar{abs}})$$
$$r^2 = .9994$$

Correcting for Tubidity

Glacial lakes, in particular, are characterized by large concentrations of suspended silt particles causing turbidity (>5-10 NTU). In the analysis of total iron (TFe), the turbidity of unfiltered water could elevate sample absorbances causing artificially high values. However, when we compared

estimates of iron made without turbidity blanks (TFe) to iron levels found with turbidity blanks (NFe), we found an error of only 1% (Table 11). For the range in turbidity and TFe tested, no turbidity correction is warranted.

Table 11. Comparisons of total iron (TFe) to turbidity corrected iron (NFe) showing an ~1% turbidity error when using the manual analysis, and turbidity (NTU) levels in lakes influenced by glacial silt.

Lake	Turbidity (NTU)	TFe ($ug\ L^{-1}$)	NFe ($ug\ L^{-1}$)
Silver	2	120	118
Kenai	4	200	198
Coghill	8	633	628
Upper Trail	20	1,389	1,365
Upper Trail	27	1,809	1,788
Tustumena	30	2,096	2,075
Tustumena	36	2,691	2,655
Tustumena	42	3,073	3,033

$NFe = 0.4893 + 0.9871\ TFe$

$r^2 = 1.000$

Total Iron (automated, tentative)

This method is adapted from Skougstand et al. (1979). The chemical principles are the same as those described for the manual method.

Standard Range

5-500 $ug\ L^{-1}$ Fe.

Lower Limit of Detection

Empirical: 5 $ug\ L^{-1}$ Fe.
Predicted: Not available.

Apparatus

Autoanalyzer II equipped with 520-nm filters and 50-mm flow cells, autoclave (121 C, 15 psi), and 30-ml culture tubes with autoclavable caps.

Reagents

1) Ferrozine (iron reagent) - Premixed solution commercially available from Hach Chemical Company.

2) Hydroxylamine-hydrochloric acid - Dissolve 100 g of hydroxylamine hydrochloride into ~500 ml of DI water containing 40 ml of concentrated HCl, and dilute to 1 liter with DI water.

3) Sodium acetate solution - Dissolve 250 g of sodium acetate-trihydrate into ~750 ml of DI water, and dilute to 1 liter.

4) Sample wash - Add 0.5 ml of Brij 35 wetting agent (Technicon Industries) per liter of DI water.

Procedure

1) Pour 10 ml of unfiltered sample or standard into a 30-ml culture tube, and add 0.3 ml of hydroxylamine-hydrochloric acid.

2) Cover the tube and digest in the autoclave (121 C, 15 psi) for 15 minutes.

3) Cool, and place a 4-ml aliquot into the sampler tray.

4) Arrange the Autoanalyzer (Figure 12), baseline, calibrate (see Instrument Calibrations, p. 192), and pump the standards and samples through the system.

Standards

Primary iron standard (50 ug ml^{-1} Fe) - Dissolve 0.0605 g of ferric chloride -6-hydrate ($FeCl_3$-$6H_2O$) into ~200 ml of DI water containing 2.5 ml of 1:1 sulfuric acid, and dilute to 250 ml with DI water.

Secondary iron standard (0.5 ug ml^{-1} Fe) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample chart deflections, and substitute into the regression formula (Table 12).

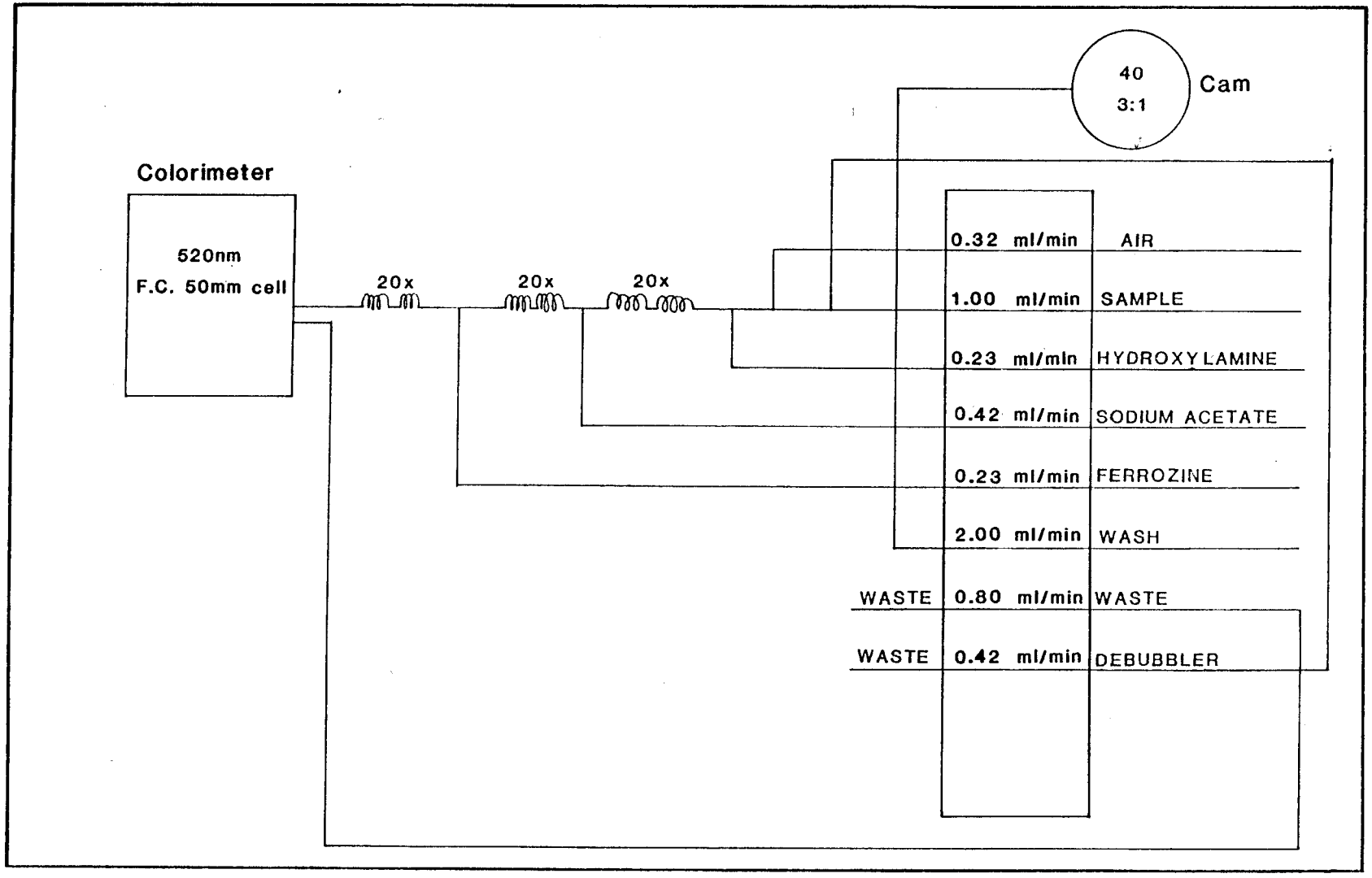

Figure 12. Autoanalyzer schematic showing reagent lines with flow rates (ml/min), cam size (number of samples per hour and sample to wash ratio), mixing coils, and colorimeter with specified flow cell (FC) and wavelength (nm) used in the analysis of total iron.

Table 12. The relationship between known concentrations of iron and autoanalyzer chart deflections ($\bar{cd}$) used to calculate a standard curve in the automated analysis of total iron.

Secondary standard (ml)	Total volume (L)	Iron concentration (ug L^{-1})	Chart deflection (x)	$\bar{cd}$ ($\bar{x}$-blank)
0.0	.010	0	5.5, 5.0	0.0
0.1	.010	5	6.0, 6.0	0.2
0.2	.010	10	6.5, 7.0	1.0
1.0	.010	50	14.0, 14.0	8.2
2.0	.010	100	25.0, 25.0	19.2
10.0	.010	500	98.0, 98.0	92.2

$$Fe\ (ug\ L^{-1}) = 2.3553 + 5.3880\ (\bar{cd})$$
$$r^2 = .9997$$

Hardness

Total hardness is computed as the sum of the cations contributing to hardness and is expressed as equivalent concentrations of calcium carbonate ($CaCO_3$). To calculate hardness, multiply the concentrations of the hardness producing cations by the factors listed in Table 13 and sum to obtain total hardness (mg L^{-1} as $CaCO_3$). Report the results in terms of the cations involved.

Table 13. Factors used to express various metal cations as equivalent concentrations of $CaCO_3$.

Cation	Factor
Ca	2.497
Mg	4.116
Fe	1.792
Mn	1.822
Al	3.710
Zn	1.531
Sr	1.142

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Strickland, J. D. H. and T. R. Parsons. 1972. A practical handbook of seawater analyses. Bull. Fish. Res. Board Canada 167. 311 p.

Hardness

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DISSOLVED GASES

Dissolved Oxygen

This method describes the Winkler procedure for dissolved oxygen (D.O.) determination.

Range

0.1-20.0 mg L^{-1}.

Limit of Detection

0.5 mg L^{-1}.

Apparatus

10-ml automatic buret (0.1 ml divisions), 300-ml BOD bottles, and 250-ml erlenmeyer flasks.

Reagents

1) Manganese sulfate solution (I) - Dissolve 365 g $MnSO_4H_2O$ into ~500 ml DI of water, and dilute to 1 liter.

2) Alkaline-iodide solution (II) - (1) Dissolve 500 g of NaOH into ~400 ml of DI water and dilute to 500 ml, and (2) in a mixing cylinder dissolve 300 g of potassium iodide in ~300 ml of DI water, and dilute to 450 ml. Carefully mix parts 1 and 2 as the mixture will get hot.

3) Sulfuric acid - Concentrated reagent-grade H_2SO_4.

4) 2% starch solution - Suspend 2 g of soluble starch in 300-400 ml of DI water. Add an ~20% NaOH solution while stirring until the starch mixture becomes clear, and dilute to 1 liter with DI water.

5) 0.025 N sodium thiosulfate - Dissolve 6.20 g of sodium thiosulfate into ~500 ml of DI water, and dilute to 1 liter.

Procedure

1) Collect the sample in a 300-ml BOD bottle without trapping air bubbles in the bottle (see Dissolved Gases, p. 8).

2) Add in order 2 ml each of solutions I and II. Invert several times to mix.

3) Allow the floc or precipitate to settle, mix again, and allow to resettle.

4) Add 2 ml of sulfuric acid and mix until the floc is completely dissolved. The sample is now fixed and can be analyzed later (<8 hr) if kept in the dark.

5) Pour 101 ml of the fixed sample into a 250-ml erlenmeyer flask.

6) Using an automatic buret, titrate the sample with 0.025 N sodium thiosulfate to a pale straw color.

7) Add 1-2 ml of the starch solution and complete the titration until the blue-black color turns clear. Record the volume (ml) of titrant used.

Calculations

1) For a 101 ml sample, 1 ml of 0.025 N sodium thiosulfate equals 2 mg L^{-1} of D.O.; i.e., volume of titrant (ml) x 2 = mg L^{-1} of D.O.

2) For a 203 ml sample, 1 ml of 0.025 N sodium thiosulfate equals 1 mg L^{-1} of D.O.; i.e., volume of titrant (ml) x 1 = mg L^{-1} of D.O.

Hydrogen Sulfide

Sulfur is a nutrient required by phytoplankton. However, when present as hydrogen sulfide (H_2S), it is of even greater interest in lake studies owing to its toxicity to aquatic life. H_2S is produced by the decomposition of organic material under anaerobic conditions. It exists in equilibrium with the nontoxic dissociated ion HS^{-1} according to the reaction $H_2S \longleftrightarrow H^+ + HS^{-1}$. This reaction is pH dependent as the concentration of H_2S increases with a decrease in pH. Therefore, it is important to determine in-situ pH when investigating sulfide levels. H_2S concentrations in excess of 3 ug L^{-1} are considered harmful to salmonids (Table 14).

This procedure is modified from Strickland and Parsons (1972) and Cline (1969). Sulfide is converted to a violet-colored compound by its reaction with p-phenylenediamine and ferric chloride.

Range

1.5-10,000 ug L^{-1} S.

Limit of Detection

Empirical: 1.5 ug L^{-1} S.

Apparatus

Spectrophotometer (600 nm), 50-ml stoppered cylinders, and 10-mm cuvettes.

Reagents

1) P-phenylenediamine-HCl - Dissolve 1 g of p-phenylene-diamine into a mixture of 100 ml of concentrated HCl and 300 ml of DI water, cool, and dilute to 500 ml with DI water.

2) Ferric chloride solution - Dissolve 16.6 g of ferric chloride-6-hydrate ($FeCl_3$-$6H_2O$) into 100 ml of concentrated HCl, carefully add to ~450 ml of DI water, and dilute to 500 ml with DI water.

Procedure

1) Collect a 50 ml sample in a 300-ml BOD bottle without trapping air bubbles inside (see Dissolved Gases, p. 8).

2) Pour 50 ml of sample into a 50-ml stoppered cylinder, and immediately add 5 ml of the p-phenylenediamine-HCl solution and invert to mix.

3) Within 2 minutes, add 1 ml of the ferric chloride solution and invert to mix.

4) After two minutes invert again. Color development is complete within 30 minutes and is stable for days. However, keep the cylinders cool and in the dark until they can be analyzed.

   NOTE: Prepare replicate reagent blanks from source water known to be free of sulfide.

5) Measure the absorbance against reagent blanks at 600 nm (see Instrument Calibrations, p. 192).

Calculations

Calculate the concentration of total sulfide (TS) using the expression:

$$TS\ (ug\ L^{-1}) = As - Ab \times 1168$$

As = Sample absorbance
Ab = Blank absorbance

To determine if toxic levels of H_2S are present, find the proportion of H_2S based on the sample pH using Table 14, and multiply by the concentration of total sulfide.

Table 14. The proportions of H_2S and HS^{-1} as a function of pH.

pH	H_2S	HS^{-1}
5.0	1.00	0.00
6.0	0.90	0.10
6.4	0.80	0.20
6.6	0.70	0.30
6.8	0.60	0.40
7.1	0.50	0.50
7.2	0.40	0.60
7.4	0.30	0.70
7.6	0.20	0.80
8.0	0.10	0.90
9.0	0.00	1.00

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Reactive Silicon (manual)

Silicon is a primary nutrient required by diatoms for the formation of frustules. Should reactive silicon levels become diminished, diatoms may be replaced by other algae.

This method measures reactive silicon, presumably the inorganic form available for algal uptake. Silicon reacts with ammonium molybdate to form a yellow-colored silicomolybdate complex which is reduced by sodium sulfite to produce an intense blue color.

Standard Range

100-4000 ug L^{-1} Si.

Upper Limit of Detection

5000 ug L^{-1} Si.

Lower Limit of Detection

Empirical: 20 ug L^{-1} Si.
Predicted: 27 ug L^{-1} Si.

Precision

6% at 1868 ug L^{-1} Si.

Accuracy

$\pm$ 2% at 1868 ug L^{-1} Si.

Apparatus

Spectrophotometer (810 nm) wavelength, 15-ml disposable centrifuge tubes, and 10-mm cuvettes.

Reagents

1) Ammonium molybdate - Dissolve 2.0 g of ammonium molybdate-4-hydrate in ~150 ml of DI water. Add 6 ml of concentrated hydrochloric acid, and dilute to 250 ml with DI water. Store in a polyethylene wash bottle and discard when a precipitate forms.

2) Metol-sulfite - Dissolve 2.4 g of sodium sulfite-anhydrous into 200 ml of DI water. Add 4 g of

p-methylaminophenol sulfate (metol) and dissolve. Prepare fresh daily.

3) Oxalic acid - Dissolve 10 g of oxalic acid into ~80 ml of DI water, and dilute to 100 ml.

4) 1:1 sulfuric acid - Slowly pour 250 ml of concentrated H_2SO_4 into ~200 ml of DI water, cool, and dilute to 500 ml.

5) Reducing reagent - Combine 167 ml of metol-sulfite, 100 ml of oxalic acid, 100 ml of 1:1 sulfuric acid, and 133 ml of DI water.

Procedure

1) Place 5 ml of unfiltered sample or standard into a 15-ml centrifuge tube.

2) Add 2 ml of molybdate reagent, invert to mix, and allow 15 minutes for color (yellow) development.

3) Add 3 ml of reducing reagent, invert 3 times, and allow 2 hours for full color (blue) development.

4) Measure the absorbance at 810 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

1) Primary standard (0.467 mg ml^{-1} Si) - Dissolve 0.782 g of sodium fluosilicate (Na_2SiF_6) into ~200 ml of DI water, and dilute to 250 ml.

2) Secondary standard (4.67 ug ml^{-1}) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against the averaged absorbances minus the blank (X-axis) and calculate the coefficient of determination (r^2).

2) Calculate sample concentration by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 15).

Table 15. The relationship between known concentrations of reactive silicon and absorbances ($\bar{\bar{abs}}$) at 810 nm used to calculate a standard curve in the manual analysis of reactive silicon.

Secondary standard (ml)	Total volume (L)	Reactive silicon concentration (ug L^{-1})	Absorbance (x)	$\bar{\bar{abs}}$ ($\bar{x}$-blank)
0.0	.005	0	.001, .001	.000
0.1	.005	93	.044, .042	.042
1.0	.005	934	.401, .415	.407
2.0	.005	1,868	.796, .812	.803
3.0	.005	2,802	1.164,1.205	1.184
4.0	.005	3,736	1.573,1.593	1.582

$$Si\ (ug\ L^{-1}) = -12.6923 + 2366.6386\ (\bar{\bar{abs}})$$

$$r^2 = .9999$$

Correcting for Turbidity

Glacial lakes, in particular, are characterized by large concentrations of suspended silt particles causing turbidity (>5-10 NTU). In the manual analysis for reactive silicon (RSi), the turbidity of unfiltered water could elevate sample absorbances causing artificially high values. However, when we compared estimates of RSi made without turbidity blanks to those made with turbidity blanks (NRSi), we found an error of only 0.6% (Table 16). For the range in turbidities and RSi tested, no turbidity correction is warranted.

Table 16. Comparison of reactive silicon (RSi) levels to turbidity corrected silicon (NRSi) showing <1% turbidity error when using the manual procedure. Also shown are RSi levels determined by the automated procedure, and a range of turbidity levels (NTU) within Alaskan lakes.

Lake	Turbidity (NTU)	RSi (manual) (ug L^{-1})	NRSi (manual) (ug L^{-1})	RSi (automated) (ug L^{-1})
Silver	1	1,165	1,159	1,242
Crosswind	1	1,025	1,025	1,172
Miners	3	996	996	1,085
Klutina	5	1,425	1,425	1,556
Kenai	6	1,596	1,596	1,853
Skilak	10	1,525	1,525	1,705
Tazlina	29	1,549	1,524	1,722
Miners	33	1,000	959	1,093
Tonsina	40	1,656	1,602	1,792
Upper Trail	44	1,836	1,790	1,966
Tustumena	46	2,364	2,312	2,499
Petrof	68	621	510	666

NRSi (manual) = -36.9087 + 1.0064 RSi (manual)

r^2 = .9947

Reactive Silicon (automated)

This method is adapted from Technicon Industrial Systems (1977). Ascorbic acid is the reducing agent used to convert silicomolybdates to molybdenum-blue compounds.

Standard Range

100-1750 ug L^{-1} Si.

Upper Limit of Detection

3000 ug L^{-1} Si.

Lower Limit of Detection

Empirical: 20 ug L^{-1} Si.
Predicted: 33 ug L^{-1} Si.

Precision

17% at 583 ug L^{-1} Si.

Accuracy

$\pm$ 2% at 583 ug L^{-1} Si.

Apparatus

Technicon Autoanalyzer II equipped with 660-nm filters, and 50-mm flow cells.

Reagents

1) Ammonium molybdate - Dissolve 10 g of ammonium molybdate 4-hydrate in 1 liter of 0.1 N sulfuric acid.

2) Oxalic acid - Dissolve 50 g of oxalic acid into ~500 ml of DI water, and dilute to 1 liter.

3) Ascorbic acid - Dissolve 17.6 g of ascorbic acid into ~500 ml of DI water containing 50 ml of reagent grade acetone. Dilute the mixture to 1 liter with DI water, and add 0.5 ml of Levor wetting agent (Technicon Industries).

4) Sample wash - Add 1 ml of Triton-X (Technicon Industries) per liter of DI water.

Procedure

1) Arrange the autoanalyzer (Figure 13), baseline, and calibrate (see Instrument Calibrations, p. 192).

2) Place 4 ml of unfiltered sample or standard into the sampler tray and pump through the system.

Standards

Primary standard (0.7 mg ml^{-1} Si) - Dissolve 2.335 g of sodium fluosilicate (Na_2SiF_6) into ~250 ml of DI water, and dilute to 500 ml.

Secondary standard (7 ug ml^{-1} Si) - Dilute 1.0 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficient of determination (r^2).

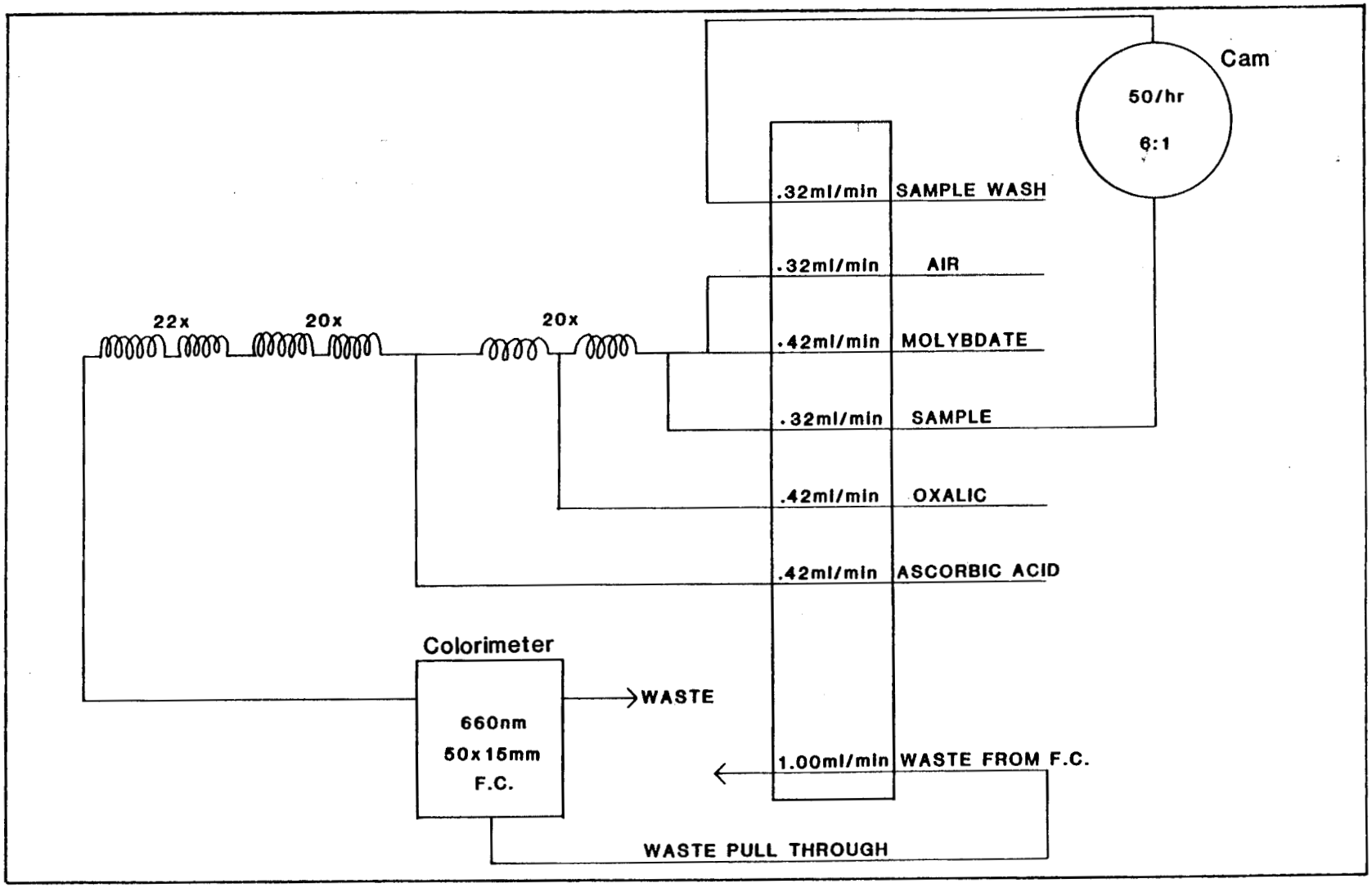

Figure 13. Autoanalyzer schematic showing reagent lines with flow rates (ml/min), cam size (number of samples per hour and sample to wash ratio), mixing coils, and colorimeter with specified flow cell (FC) and wavelength (nm) used in the analysis of reactive silicon.

2) Calculate sample concentrations by subtracting the blank value from the sample chart deflections, and substitute into the regression formula (Table 17).

NOTE: Because of possible contamination of silicon especially from borosilicate glassware use polyethylene containers for preparing and storing reagents and standards.

Table 17. The relationship between known concentrations of reactive silicon and autoanalyzer chart deflections ($\bar{c}\bar{d}$) used to calculate a standard curve in the automated analysis of reactive silicon.

Secondary standard (ml)	Total volume (L)	Reactive silicon concentration (ug L^{-1})	Chart deflection (x)	$\bar{c}\bar{d}$ ($\bar{x}$-blank)
0.00	.004	0.0	0, 1	0.0
0.05	.004	87.5	3, 5	3.5
0.10	.004	175.0	10, 11	10.0
0.30	.004	525.0	29, 31	29.5
0.50	.004	875.0	50, 51	50.0
1.00	.004	1750.0	100, 101	100.0

$$Si\ (ug\ L^{-1}) = 9.2640 + 17.3933\ (\bar{c}\bar{d})$$

$$r^2 = .9998$$

Nitrogen

In lakes, nitrogen may exist as organic nitrogen, ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-). The various inorganic forms can be used as nitrogen sources by phytoplankton; however, ammonium is preferred. Ammonium, a primary end product of organic decomposition reactions, can be oxidized to nitrite and nitrate by bacteria (nitrification) in well-oxygenated water. However, nitrate reduction (denitrification) can also occur, but only during periods of reduced oxygen. Thus, under aerobic conditions nitrate dominates the inorganic nitrogen fractions, while under low oxygen or anaerobic conditions (<2 mg l^{-1} O_2), ammonia dominates.

Total Ammonia (manual)

Ammonia exists both as the unionized form NH_3 and the ammonium ion NH_4^+. The proportion of NH_3 increases with an increase in temperature and pH, and is considered toxic to salmonids at levels above 0.016 mg L^{-1} (Willingham et al. 1979). As the pH of natural waters is typically between 5 and 8 units, NH_4^+ is present in far greater concentrations than NH_3. In addition, concentrations of total ammonia (NH_3 + NH_4^+) are thought to be harmful at levels exceeding 1.0 mg L^{-1} (Haywood 1983).

Under basic conditions total ammonia (NH_3 + NH_4^+) is converted to NH_3 and reacts with phenol and sodium hypochlorite to form blue indophenol compounds.

Standard Range

1-50 ug L^{-1} N.

Upper Limit of Detection

200 ug L^{-1} N.

Lower Limit of Detection

Empirical: 1.0 ug L^{-1} N.
Predicted: 0.25 ug L^{-1} N.

Precision

15% at 15 ug L^{-1} N.

Accuracy

± 8% at 15 ug L^{-1} N.

Apparatus

Spectrophotometer (640 nm), 50-ml stoppered cylinders, and 100-mm cuvettes.

Reagents

1) Phenol - Dissolve 25 g of crystalline phenol into ~100 ml of reagent alcohol, and dilute to 250 ml with reagent alcohol.

2) Ferrocyanide solution - Dissolve 1.55 g of potassium ferrocyanide-trihydrate into ~200 ml of DI water, and dilute to 250 ml.

3) Hypochlorite solution - Dissolve 80 g of sodium citrate-trihydrate and 4 g of sodium hydroxide into ~300 ml of DI water. Add 100 ml of Clorox (5% sodium hypochlorite), and dilute to 500 ml with DI water.

Procedure

1) Pour 50 ml of sample or standard into a 50-ml stoppered cylinder.

2) Add 2 ml of phenol and invert to mix.

3) Add 2 ml of potassium ferrocyanide and invert to mix.

4) Add 5 ml of the hypochlorite solution, invert twice to mix, and after 15 minutes invert again.

5) Allow 2 hours for full color development, and measure the absorbance at 640 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary nitrogen standard (0.2 mg ml^{-1} N) - Dissolve 0.3824 g of ammonium chloride (NH_4Cl) into ~400 ml of DI water, and dilute to 500 ml.

Secondary standard (0.5 ug ml^{-1} N) - Dilute 0.25 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 18).

Table 18. The relationship between known concentrations of nitrogen and absorbances ($\bar{abs}$) at 640 nm used to calculate a standard curve in the manual analysis of total ammonia.

Secondary standard (ml)	Total volume (L)	Nitrogen concentration (ug L^{-1})	Absorbance (x)	$\bar{abs}$ ($\bar{x}$-blank)
0.0	.050	0	.062, .060	.000
0.1	.050	1	.072, .077	.014
0.3	.050	3	.096, .099	.037
0.5	.050	5	.121, .125	.062
1.5	.050	15	.234, .251	.182
5.0	.050	50	.648, .639	.583

$$N\ (ug\ L^{-1}) = -.0.2463 + 85.9655\ (\bar{abs})$$

$$r^2 = .9999$$

3) Determine the concentration of toxic NH_3 by finding the percentage of NH_3, based on in situ temperature and pH (Table 19), dividing by 100, and multiplying by the concentration of total ammonia.

Table 19. The percent unionized ammonia (NH_3) in aqueous ammonia + ammonium (NH_4+) solutions (after Emerson et al. 1975).

Temp.	pH								
(C)	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	.01	.03	.08	.30	0.8	2.6	7.6	20.7	45.3
5	.01	.04	.13	.39	1.2	3.8	11.1	28.3	55.6
10	.02	.06	.19	.59	1.8	5.6	15.7	37.1	65.1
15	.03	.09	.27	.86	2.7	8.0	21.5	46.4	73.7
20	.04	.13	.40	1.2	3.8	11.2	28.4	55.7	79.9

Example

Fred Lake with a total ammonia concentration of 30 ug L^{-1}, a temperature of 10 C, and a pH of 7.5 has a toxic NH_3 level of 0.18 ug L^{-1}.

Total Ammonium (automated)

This procedure is a modification of Industrial Method No. 154-71W/B (Technicon Industrial Methods 1978). The autoanalyzer arrangement is identical to the Technicon method, except for reducing the sampling rate to 30/hr and using sodium nitroferrocyanide as the catalyst. The chemistry involved is the same as that described in the manual method, and the procedure can be run simultaneously with the analysis of nitrate and nitrite nitrogen.

Standard Range

12.5-100 ug L^{-1} N.

Upper Limit of Detection

500 ug L^{-1} N.

Lower Limit of Detection

Empirical: 1 ug L^{-1} N.
Predicted: 1.1 ug L^{-1} N.

Precision

13% at 20 ug L^{-1} N.

Accuracy

$\pm$ 7% at 20 ug L^{-1} N.

Apparatus

Technicon Autoanalyzer II equipped with 630-nm filters and 50-mm flow cells.

Reagents

1) Complexing reagent - Dissolve 33 g of potassium sodium tartrate, and 24 g of sodium citrate-dihydrate in ~950 ml of DI water. Adjust to a pH of 5.0 with ~2 ml of concentrated sulfuric acid, and dilute to 1 liter with DI water. Add 0.5 ml of Brij 35 wetting agent (Technicon Industries).

2) Phenol - Dissolve 83 g of phenol in 50 ml of DI water. Slowly add 180 ml of 20% sodium hydroxide (50 g of NaOH diluted to 250 ml with DI water). Dilute the mixture to 1 liter with DI water.

3) Hypochlorite solution - Dilute 200 ml of Clorox bleach (5% hypochlorite) to 1 liter with DI water.

4) Catalyst - Dissolve 0.55 g of sodium nitroferrocyanide-dihydrate in ~750 ml of DI water, and dilute to 1 liter.

5) Sample wash - Add 0.5 ml of Brij 35 per liter of DI water.

Procedure

1) Arrange the autoanalyzer (Figure 14), baseline, and calibrate (see Instrument Calibrations, p. 192).

2) Place 4 ml of filtered sample or standard into the sampler tray and pump through the system.

Standards

Primary nitrogen standard (0.5 mg ml^{-1} N) - Dissolve 0.9542 g of ammonium chloride (NH_4Cl) in 250 ml of DI water, and dilute to 500 ml.

Secondary standard (1.25 ug ml^{-1} N) - Dilute 0.5 ml of the primary standard to 200 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample chart deflections, and substitute into the regression formula (Table 20).

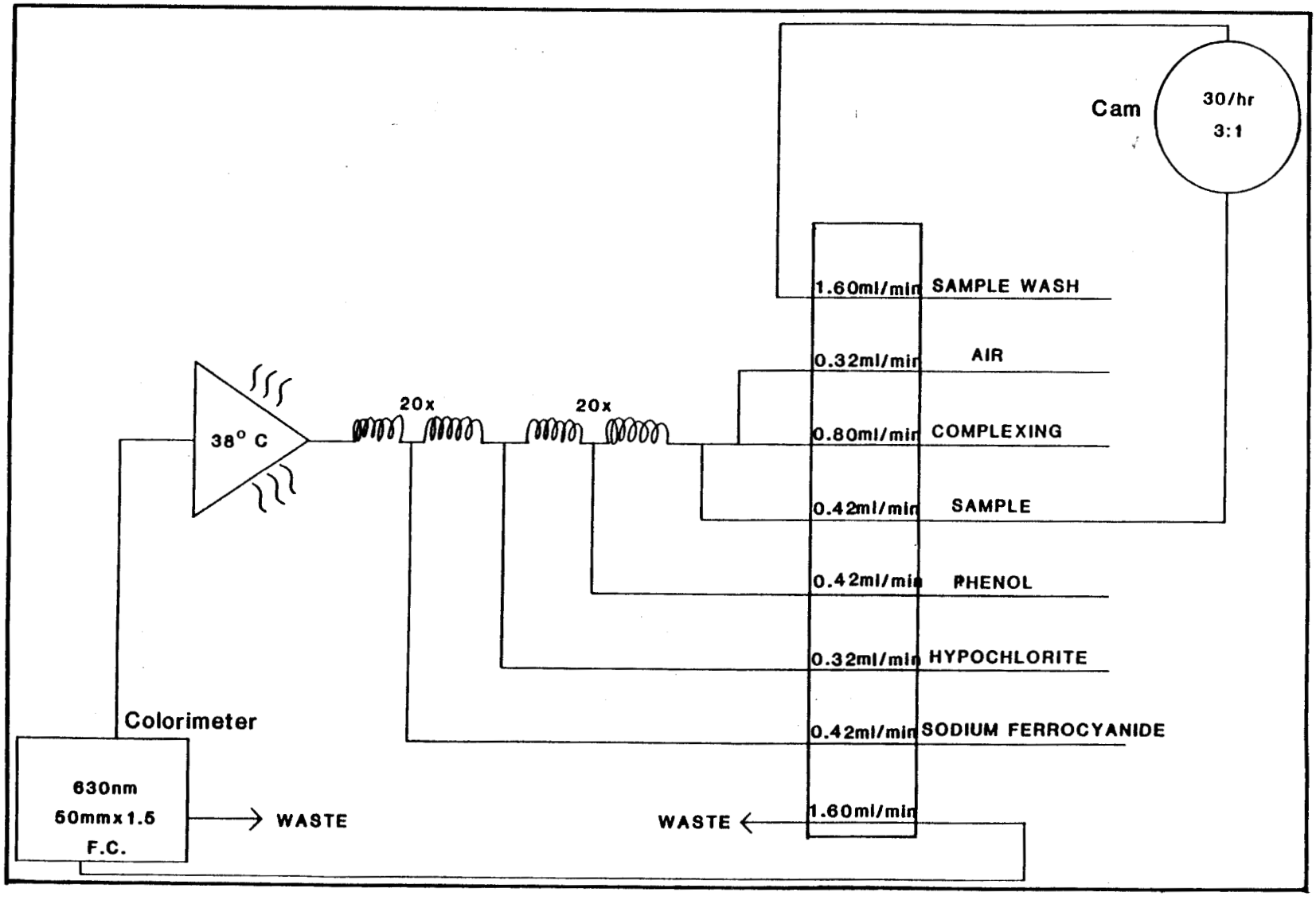

Figure 14. Autoanalyzer schematic showing reagent lines with flow rates (ml/min), cam size (number of samples per hour and sample to wash ratio), mixing coils, heating bath, and colorimeter with specified flow cell (FC) and wavelength (nm) used in the analysis of total ammonia.

Table 20. The relationship between known concentrations of nitrogen and autoanalyzer chart deflections ($\bar{cd}$) used to calculate a standard curve in the automated analysis of total ammonia.

Secondary standard (ml)	Total volume (L)	Nitrogen Concentration (ug L^{-1} N)	Chart deflections (x)	$\bar{cd}$ ($\bar{x}$-blank)
0.00	.005	0.0	3.0, 3.0	0.0
0.05	.005	12.5	10.0, 10.0	7.0
0.10	.005	25.0	18.0, 28.5	25.3
0.20	.005	50.0	52.5, 53.0	49.8
0.30	.005	75.0	79.0, 79.0	76.0
0.40	.005	100.0	102.5,104.0	100.3

$$N \text{ (ug } L^{-1}) = 2.0053 + 0.9693 (\bar{cd})$$

$$r^2 = .9971$$

Nitrate and Nitrite (manual)

Nitrate (NO_3^-) is a primary nitrogen source for phytoplankton. In oligotrophic systems, nitrite (NO_2^-) is oxidized to nitrate (nitrification) which is assimilated, after enzyme catalyzed reduction, during photosynthesis. Denitrification ($NO_3^- \longrightarrow NO_2^- \longrightarrow NH_3$) is often characteristic of eutrophic systems during periods of low-oxygen concentrations. As nitrite is an intermediary in both reactions, its concentration is usually insignificant.

This procedure measures nitrate plus nitrite. Nitrate is reduced to nitrite by passing a sample through a column containing cadmium granules coated with copper sulfate. Nitrite passes through unchanged. Nitrite forms a pink azo dye with sulfanilamide and NNED (Marshall's reagent) which is measured colorimetrically. Nitrite is determined on a separate sample aliquot by omitting the reduction step.

Standard Range

2-200 ug L^{-1} N.

Upper Limit of Detection

1250 ug L^{-1} N.

Lower Limit of Detection

Empirical: 1 ug L^{-1} N.
Predicted: 0.6 ug L^{-1} N.

Precision

7% at 50 ug L^{-1} N.

Accuracy

± 2% at 50 ug L^{-1} N.

Apparatus

Spectrophotometer (543 nm), 80-ml cadmium reduction columns, 50-ml stoppered cylinders, and 10-mm cuvettes.

Reagents

1) Buffer solution - Dissolve 100 g of ammonium chloride (NH_4Cl), 20 g of sodium borate-10-hydrate, and 1 g of Na-EDTA in ~750 ml of DI water, and dilute to 1 liter.

2) Sulfanilamide - Dissolve 6 g of sulfanilamide in 300 ml of DI water and 100 ml of concentrated HCl, and dilute to 500 ml with DI water.

3) NNED - Dissolve 0.6 g of N-1 naphthylethylenediamine dihydrochloride in ~250 ml of DI water, and dilute to 500 ml.

4) Cupric sulfate - Dissolve 25 g of cupric-sulfate 5-hydrate in ~400 ml of DI water and dilute to 500 ml.

5) 10% Hydrochloric acid - Slowly add 5 ml of concentrated HCl to 45 ml of DI water.

6) Cadmium - 40 to 60 mesh granules.

Procedure

1) Clean and repack the cadmium columns (Figure 15) according to the procedure described below.

2) Check the reduction efficiencies of the cadmium columns according to the procedure described below.

3) Pour 50 ml of filtered sample or standard into a 50-ml stoppered cylinder, add 5 ml of buffer solution and invert to mix.

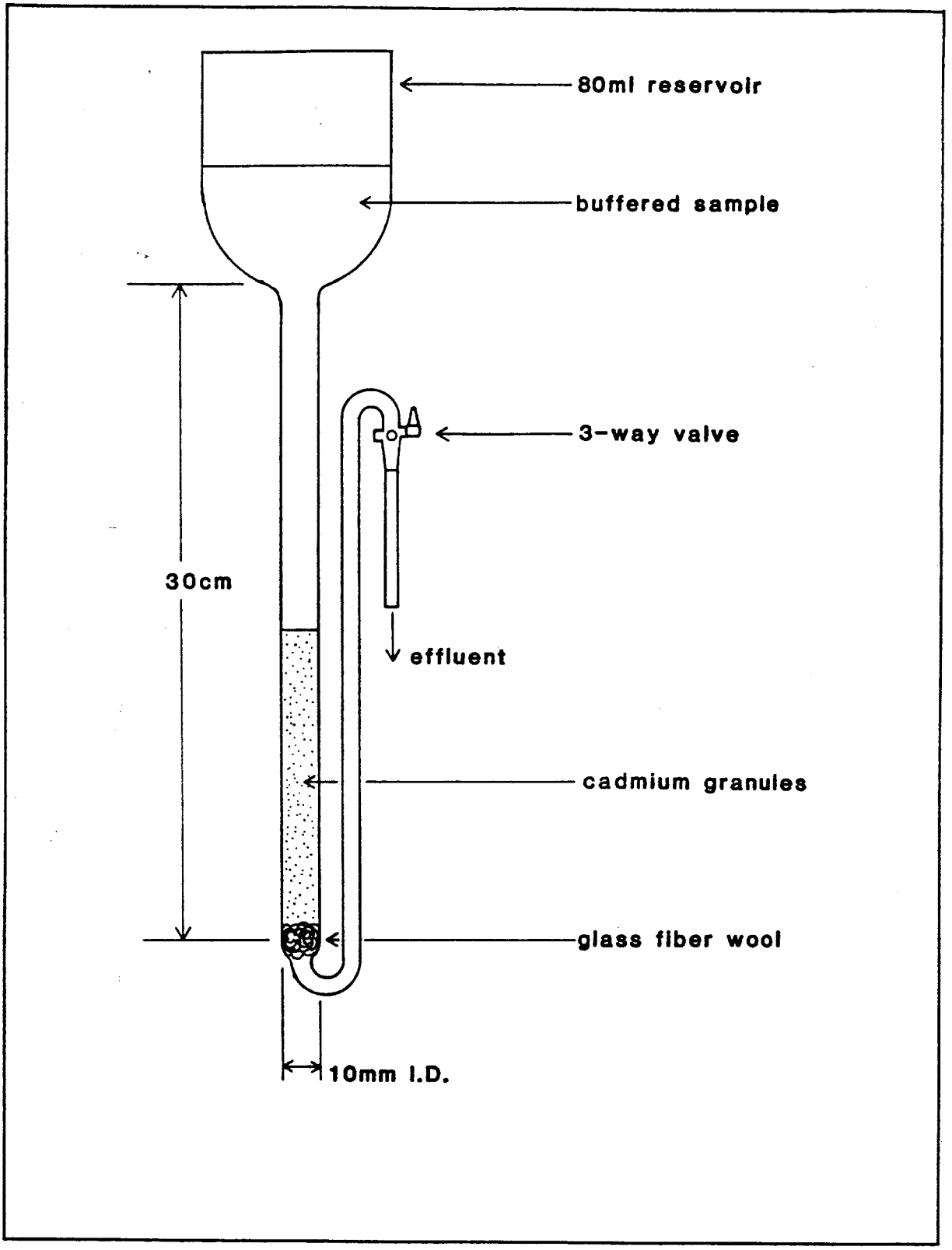

Figure 15. Cadmium column used to reduce nitrate (NO_3^-) to nitrite (NO_2^-) in the manual analysis of nitrate + nitrite nitrogen.

4) Pour 20 ml of buffered sample into the column reservoir, allow the sample to drip through, and discard the effluent.

5) 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.

6) Add 0.5 ml of sulfanilamide to the 30-ml sample, and invert twice to mix. Let sit for 5 minutes.

7) Add 0.5 ml of NNED solution, invert twice to mix, and allow 15 minutes for full color development.

8) Measure the absorbance at 543 nm against a DI water blank (see Instrument Calibrations, p. 192).

A. Cleaning and Repacking Cadmium Columns

1) Remove the cadmium granules by inverting the column into a 100-ml plastic beaker and tapping lightly.

2) Add 50 ml of 10% HCl to the cadmium, swirl, and soak for about 30 minutes.

3) Rinse the cadmium with DI water, add ~30 ml of cupric sulfate, and soak for 15 minutes.

4) Pour off the solution, rinse the cadmium with DI water, and add 30 ml of cupric sulfate. Soak until the cadmium is coated with copper; i.e., when the blue color disappears. Rinse the cadmium thoroughly with DI water.

5) 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.

6) 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.

Standards

Primary nitrate standard (0.1 mg ml^{-1} N) - Dissolve 0.36101 g of potassium nitrate (KNO_3) in ~400 ml of DI water, and dilute to 500 ml.

Secondary nitrate standard (1 ug ml^{-1} N) - Dilute 2.5 ml of the primary standard to 250 ml.

Primary nitrite standard (0.1 mg ml^{-1} N) - Dissolve 0.24630 g of sodium nitrite ($NaNO_2$) in ~400 ml of DI water, and dilute to 500 ml.

Secondary nitrite standard (1 ug ml^{-1} N) - Dilute 1 ml of the primary standard to 100 ml.

B. Reduction Efficiency

Prior to running the samples and/or standards through the columns, determine the reduction efficiencies. If the efficiencies are greater or equal to 95%, no correction of the sample absorbances is necessary as essentially all of the nitrate present in the sample has been reduced. If the efficiencies are less than 95% or over 105% repeat the cleaning and repacking procedure. However, the sample absorbances can be divided by the reduction efficiency of the appropriate column, and the corrected values used in the subsequent calculations. The efficiency procedure should be conducted for each column (Table 21).

1) Prepare a reagent blank by adding 5 ml of buffer solution to 50 ml of DI water in a stoppered cylinder.

2) Prepare two nitrate standards by diluting 10 ml of the secondary standard (1 ug ml^{-1} N) to 50 ml with DI water, and add 5 ml of buffer.

3) Prepare a nitrite standard by diluting 10 ml of the secondary standard (1 ug ml^{-1} N) to 50 ml with DI water, and add 5 ml of buffer.

4) Pour the standards through the column in the following order: blank, nitrate, nitrite, and nitrate.

Calculate the reduction efficiency (R.E.) of each column using the following:

$$R.E. = \frac{\text{(averaged absorbances of } NO_3^- \text{ standards) - blank}}{\text{(absorbance of } NO_2^- \text{ standard) - blank}}$$

Table 21. Absorbances for nitrite and equivalent nitrate standards after cadmium reduction used to calculate the reduction efficiency (R.E.) in the manual analysis of nitrate + nitrite nitrogen.

Column I			Column II	
	Concentration ($\mu g\ L^{-1}$ N)	Absorbance (543 nm)		Absorbance (543 nm)
Blank	0	0.006	Blank	0.008
Nitrate	500	1.460	Nitrate	1.483
Nitrite	500	1.550	Nitrite	1.564
Nitrate	500	1.493	Nitrate	1.501
	R.E. = 95%			R.E. = 96%

Column III		Column IV		Column V	
	Absorbance (543 nm)		Absorbance (543 nm)		Absorbance (543 nm)
Blank	0.005	Blank	0.008	Blank	0.008
Nitrate	1.489	Nitrate	1.517	Nitrate	1.483
Nitrite	1.549	Nitrite	1.542	Nitrite	1.560
Nitrate	1.494	Nitrate	1.514	Nitrate	1.508
R.E. = 96%		R.E. = 99%		R.E. = 96%	

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 22).

Table 22. The relationship between known concentrations of nitrate nitrogen and absorbances ($\bar{\bar{\bar{abs}}}$) at 543 nm following cadmium reduction used to calculate a standard curve in the manual analysis of nitrate + nitrite nitrogen.

Secondary standard (ml)	Total volume (L)	Nitrogen concentration (ug L^{-1} N)	Absorbance (x)	$\bar{\bar{\bar{abs}}}$ ($\bar{x}$-blank)
0.0	.050	0	0.025,0.025	0.000
0.1	.050	2	0.033,0.033	0.008
1.0	.050	20	0.096,0.096	0.071
2.5	.050	50	0.187,0.193	0.165
20.0	.050	400	1.234,1.218	1.201

$$N\ (ug\ L^{-1}) = -2.2895 + 334.5656\ (\bar{\bar{\bar{abs}}})$$

$$r^2 = .9998$$

Nitrate and Nitrite (automated)

This method is adapted from Stainton et al. (1977), and involves the same principles described in the manual method. This procedure can be run simultaneously with the analysis of ammonia nitrogen.

Standard Range

20.0-100 ug L^{-1} N.

Upper Limit of Detection

500 ug L^{-1} N.

Lower Limit of Detection

Empirical: 1 ug L^{-1} N.
Predicted: 3.4 ug L^{-1} N.

Precision

9% at 50 ug L^{-1} N.

Accuracy

$\pm$ 3% at 50 ug L^{-1} N.

Apparatus

Technicon Autoanalyzer II equipped with 540-nm filters, 15-mm flow cells, and cadmium reductor column.

Reagents

1) Prepare reagents 1-6 as described in the nitrate + nitrite manual method (p. 85).

2) Sample wash - Add 1 ml of Brij 35 wetting agent (Technicon Industries) per liter of DI water.

Procedure

1) Clean the cadmium granules (see p. 87) and repack the reductor column (see below).

2) Arrange the autoanalyzer (Figure 16), baseline, and calibrate the system (see Instrument Calibrations, p. 192).

3) Determine the efficiency of the reductor column (see below).

4) Place 4 ml of filtered sample or standard into the sampler tray and pump through the system.

A. Preparation of Reductor Column

Sift ~25 g of clean 40-60 mesh cadmium granules through a fine mesh screen. Using a 140-mm length of 4-mm i.d. glass tubing, place a small piece of glass fiber wool in one end, cap with a plastic nipple, and fill with DI water. Add the cadmium to a small weighing pan and rinse the granules into the tube with a DI wash bottle. Place another piece of glass wool in the opposite end, and cap with the same sized nipple.

Standards

Primary nitrate standard (0.1 mg ml^{-1} N) - Dissolve 0.3611 g of potassium nitrate (KNO_3) in ~400 ml DI water, and dilute to 500 ml.

Secondary nitrate standard (1.0 ug ml^{-1} N) - Dilute 1 ml of the primary standard to 100 ml.

Primary nitrite standard (0.1 mg ml^{-1} N) - Dissolve 0.2464 g of sodium nitrite ($NaNO_2$) in ~400 ml DI water, and dilute to 500 ml.

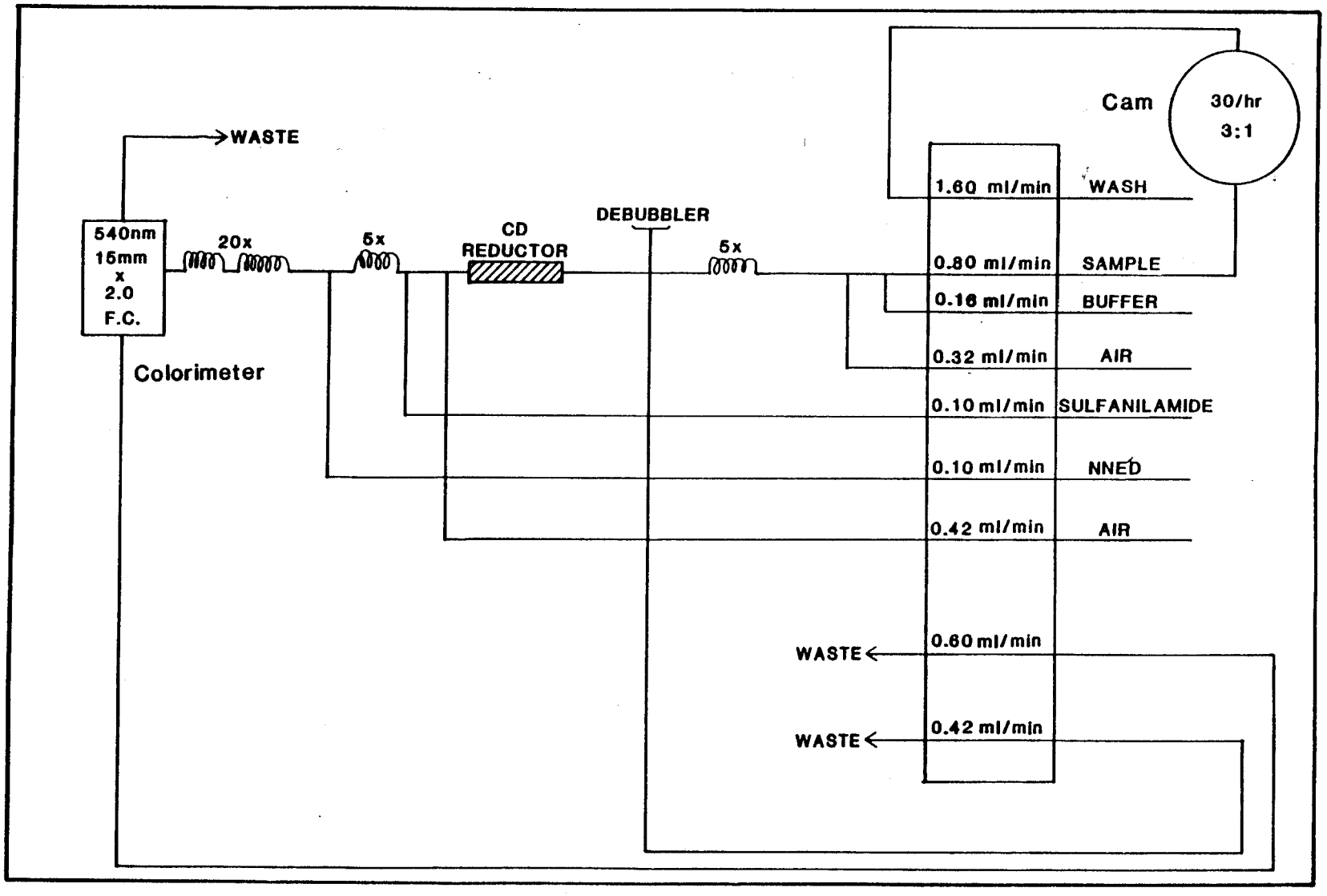

Figure 16. Autoanalyzer schematic showing reagent lines with flow rates (ml/min), cam size (number of samples per hour and sample to wash ratio), mixing coils, cadmium reduction (CD) column, debubbler, and colorimeter with specified flow cell (FC) and wavelength (nm) used in the automated analysis of nitrate + nitrite nitrogen.

Secondary nitrite standard (1.0 ug ml^{-1} N) - Dilute 1 ml of the primary standard to 100 ml.

B. Reduction Efficiency

Check the reduction efficiency (R.E.) of the column (Table 23) by preparing two nitrate standards (200 ug L^{-1}) and a nitrite standard (200 ug L^{-1}). Pump the standards through the system in the following order: blank, nitrate, blank, nitrite, blank, and nitrate. Calculate R.E. using the expression:

$$R.E. = \frac{\text{(averaged } NO_3 \text{ chart deflection) - blank}}{(NO_2 \text{ chart deflection) - blank}}$$

Table 23. Chart deflections for nitrite and equivalent nitrate standards after cadmium reduction used to calculate the reduction efficiency (R.E.) in the automated analysis of nitrate + nitrite nitrogen.

Secondary nitrate standard (ml)	Secondary nitrite standard (ml)	Concentration (ug L^{-1} N)	Chart deflection
0.0	0.0	0	2
1.0	0.0	200	100
0.0	0.0	0	3
0.0	1.0	200	99
0.0	0.0	0	3
1.0	0.0	200	101

R.E. = 101%

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank values from the sample chart deflections, and substitute into the regression formula (Table 24).

Table 24. The relationship between known concentrations of nitrate nitrogen and autoanalyzer chart deflections ($\bar{\bar{cd}}$) used to calculate a standard curve in the automated analysis of nitrate + nitrite nitrogen.

Secondary nitrate standard (ml)	Total volume (L)	Nitrogen concentration (ug L^{-1} N)	Chart deflection (x)	$\bar{\bar{cd}}$ ($\bar{x}$-blank)
0.0	.005	0.0	4.0, 2.0	0.0
0.1	.005	20.0	11.5,16.5	11.0
0.2	.005	40.0	24.5,24.0	21.0
0.3	.005	60.0	33.0,30.0	28.5
0.5	.005	100.0	50.0,50.0	47.0
1.0	.005	200.0	95.5,95.0	92.3

$$N\ (ug\ L^{-1}) = -2.9896 + 2.1919\ (\bar{\bar{cd}})$$

$$r^2 = .9992$$

Phosphorus

Phosphorus is generally accepted as the primary nutrient controlling lake productivity. Soluble orthophosphate (filterable reactive phosphorus) is the form most readily available for algal uptake; however, it usually comprises only a small portion of total phosphorus (TP). Other size fractions (colloidal, particulate, etc.) may also be present, but primarily filterable reactive and cellular (organic particulate) phosphorus are considered biologically active. All fractions should be determined individually in order to define the phosphorus cycle operating within a given lake (Figure 17). However, several of the colloidal and dissolved fractions cannot be determined directly at this time and must be derived by difference. However, our fractionation studies have shown that the sum of the individual TP components represent nearly 80% of directly determined TP (Table 25).

Definition of Phosphorus (P) Terms

TP = Total P
NTP = Total P (corrected for turbidity)
TPP = Total Particulate P
IPP = Inorganic Particulate P
OPP = Organic Particulate P
TFP = Total Filterable P
TDP = Total Dissolved P
DRP = Dissolved Reactive P
DUP = Dissolved Unreactive P
FRP = Filterable Reactive P
FUP = Filterable Unreactive P
CoRP = Colloidal Reactive P
CoUP = Colloidal Unreactive P

Total and Total Filterable Phosphorus (manual)

The method for total phosphorus (TP), and total filterable phosphorus (TFP), are adapted from the procedure described by Eisenreich et al. (1975). Phosphorus in particulate, colloidal, and organic forms is converted to inorganic orthophosphate by a persulfate-sulfuric acid digestion. Ammonium molybdate is then added to form a phosphomolybdate complex which is reduced to molybdenum-blue by ascorbic acid.

Standard Range

2-20 ug L^{-1} P.

Upper Limit of Detection

1100 ug L^{-1} P.

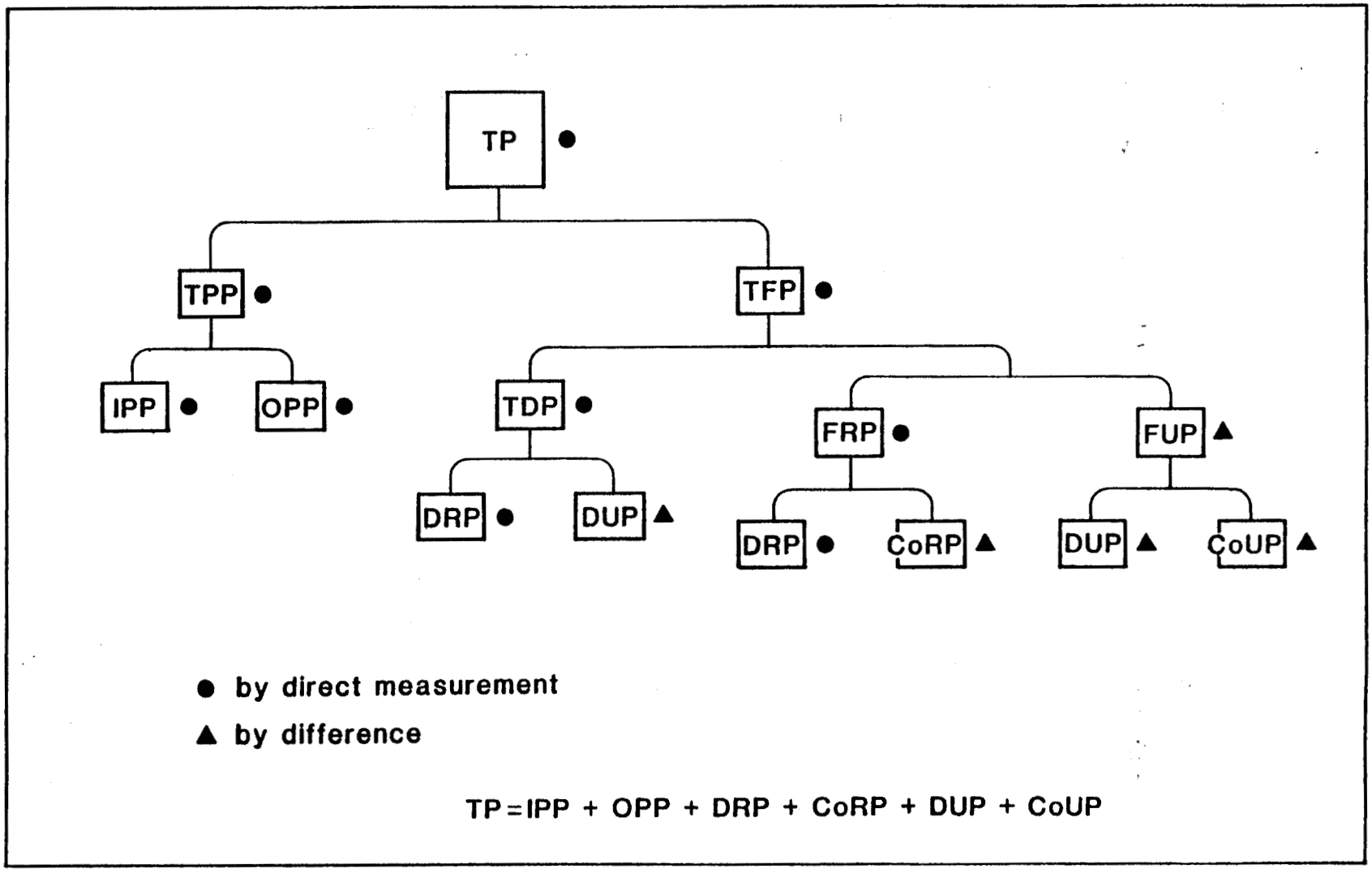

Figure 17. Phosphorus fractions comprising the total phosphorus (TP) in natural lake waters (see p. 95 for definition of terms), obtained either by direct measurement or derived by difference.

Table 25. Comparison of phosphorus levels of six fractions (see Definition of Terms) summed to give total phosphorus (TP) and directly determined TP for four lakes with varying turbidity (NTU).

Lake	NTU	IPP	OPP	DRP	CoRP	DUP	CoUP	TP (summation)	TP (direct)
Hidden	1	0.4	2.5	2.0	--	0.5	1.5	8.3	8.1
Hidden	1	0.7	2.0	2.1	--	1.5	1.3	8.1	7.2
Packers	1	1.1	5.1	1.5	2.4	0.8	4.5	15.4	13.4
Packers	1	1.8	5.0	2.1	1.3	0.8	5.3	16.3	14.6
Ptarmigan	17	9.7	3.3	1.7	--	2.8	--	17.5	19.5*
Ptarmigan	18	10.9	4.0	1.6	0.2	3.6	--	20.3	16.9*
Tustumena	50	21.8	11.8	3.9	1.0	0.8	5.2	44.5	32.8*
Tustumena	54	19.4	9.1	4.3	1.5	0.5	4.8	39.6	30.7*

*Corrected for turbidity.

Lower Limit of Detection

Empirical: 0.5 ug L^{-1} P.
Predicted: 0.25 ug L^{-1} P.

Precision

5% at 6 ug L^{-1} P.

Accuracy

$\pm$ 3% at 6 ug L^{-1} P.

Apparatus

Spectrophotometer (882 nm), autoclave (121 C, 15 psi), 50-ml erlenmeyer flasks, and 100-mm cuvettes.

Reagents

1) Antimony-tartrate solution - Add 53.3 ml of concentrated sulfuric acid (H_2SO) to ~800 ml of DI water and cool. Dissolve 0.748 g of antimony potassium tartrate trihydrate in the H_2SO_4 solution, and dilute to 1 liter with DI water. Filter the solution when a precipitate forms.

2) Molybdate solution - Dissolve 7.95 g of ammonium molybdate-4-hydrate in ~500 ml of DI water, and dilute to 1 liter.

3) 3.6 N H_2SO_4 - Add 50 ml of concentrated sulfuric acid to ~400 ml of DI water, cool, and dilute to 500 ml.

4) Digestion reagent - Dissolve 60 g of potassium persulfate in ~800 ml of DI water containing 100 ml of 3.6 N H_2SO_4, and dilute to 1 liter with DI water.

5) Mixed Reagents (MR I) - Combine 250 ml of both the antimony-tartrate and molybdate solutions. Add 2 g of ascorbic acid, dissolve, and dilute to 1 liter with DI water.

Procedure

1) Pour 25 ml of unfiltered (TP) or filtered (TFP) sample or standard in a 50-ml erlenmeyer flask.

2) Add 5 ml of digestion reagent, cover with a 15-ml polypropylene beaker, mix, and digest in the autoclave for 30 minutes.

3) Cool to room temperature, and add 5 ml of MR I and mix.

4) Allow 20 minutes for full color development and measure the absorbance at 882 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary phosphorus standard (0.05 mg ml^{-1} P) - Dissolve 0.1389 g of potassium phosphate-dibasic (K_2HPO_4) in ~400 ml of DI water, and dilute to 500 ml.

Secondary Standard (0.5 ug ml^{-1} P) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 26).

Table 26. The relationship between known concentrations of phosphorus and absorbances ($\bar{abs}$) at 882 nm used to calculate a standard curve in the analyses of total and total filterable phosphorus.

Secondary standard (ml)	Total volume (L)	Phosphorus concentration (ug L^{-1})	Absorbance (x)	$\bar{abs}$ ($\bar{x}$-blank)
0.0	.025	0	.006, .005	.000
0.1	.025	2	.015, .017	.011
0.2	.025	4	.026, .027	.021
0.3	.025	6	.036, .035	.030
0.5	.025	10	.059, .060	.054
1.0	.025	20	.109, .111	.105

$$P \text{ (ug } L^{-1}) = 0.0064 + 189.8708 \ (\bar{abs})$$

$$r^2 = .9994$$

Correcting for Turbidity

In the analysis of TP, absorbance measurements of turbid solutions are artificially high because of light backscattering (Jullander and Brune 1950). As glacial lakes, in particular, are characterized by large concentrations of suspended silt particles causing turbidity (>5-10 NTU); TP levels need to be

corrected (Table 27). The additional absorbance due to turbidity can be determined by analyzing a turbidity blank in conjunction with the sample (see Interferences p. 198). However, from a thorough analysis of glacial lakes; we found that turbidity corrected TP (NTP) can be obtained using the equation:

$$NTP\ (ug\ L^{-1}) = 0.8229 + 0.5845\ TP\ (ug\ L^{-1})$$
$$r^2 = 0.902$$

Therefore, only NTP of glacial lakes (4-12; Table 27) is comparable to TP in clear-water or non-turbid lakes (1-3; Table 27). Finally, the presence of iron concentrations above 10 mg L^{-1} inhibit color formation. This interference can be avoided by an appropriate dilution of the sample prior to digestion.

Table 27. Comparison of total phosphorus (TP-manual) levels, turbidity corrected TP (NTP), and of TP (automated) for lakes with varying turbidity (NTU).

Lake	Turbidity (NTU)	TP (manual) (ug L^{-1})	NTP (manual) (ug L^{-1})	TP (automated) (ug L^{-1})
Silver	1	6.1	5.3	7.4
Crosswind	1	8.8	8.5	--
Miners	3	11.8	12.4	14.1
Klutina	5	9.1	4.5	8.3
Kenai	6	11.1	6.2	10.2
Skilak	10	22.4	12.3	11.2
Tazlina	29	50.4	23.5	26.3
Miners	33	87.2	32.7	33.6
Tonsina	40	70.4	27.8	39.0
Upper Trail	44	88.9	33.2	35.2
Tustumena	46	75.0	41.2	46.1
Petrof	68	156.8	61.4	65.2

Filterable Reactive Phosphorus (manual)

Filterable reactive phosphorus (FRP) includes both dissolved (DRP) and colloidal reactive (CoRP) phosphorus (Figure 17). Since the proportions vary according to lake type (i.e., glacial, organically stained, and clear), determination of FRP may not accurately reflect the amount of soluble orthophosphate in the sample.

Standard Range

1-10 ug L^{-1} P.

Upper Limit of Detection

1100 ug L^{-1} P.

Lower Limit of Detection

Empirical: 0.5 ug L^{-1} P.
Predicted: 0.12 ug L^{-1} P.

Precision

7% at 4 ug L^{-1} P.

Accuracy

$\pm$ 4% at 4 ug L^{-1} P.

Apparatus

Spectrophotometer (882 nm), 50-ml erlenmeyer flasks, and 100-mm cuvettes.

Reagents

1) Prepare reagents 1-3 as described in the TP and TFP methods (see p. 98).

2) Mixed reagent II (MR II) - Combine in order, 250 ml of ammonium-molybdate solution, 100 ml of 3.6 N H_2SO_4, and 250 ml of the antimony-tartrate solution. Add 2.0 g of ascorbic acid, dissolve, and dilute to 1 liter.

Procedure

1) Pour 25 ml of filtered sample or standard into a 50-ml erlenmeyer flask.

2) Add 5 ml of MR II, and mix.

3) Allow 20 minutes for full color development, and measure the absorbance at 882 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Prepare primary and secondary standards as described in the TP and TFP method (see p. 99).

Calculations

The calculations are identical to that described in the TP and TFP method (see p. 99); however, the standard curve is not identical (Table 28) and needs to be determined separately.

Table 28. The relationship between known concentrations of phosphorus and absorbances ($\bar{abs}$) at 882 nm used to calculate a standard curve in the analysis of filterable reactive phosphorus.

Secondary standard (ml)	Total volume (L)	Phosphorus concentration (ug L^{-1})	Absorbance (x)	$\bar{abs}$ ($\bar{x}$-blank)
0.00	.000	0.0	.003, .003	.000
0.05	.025	1.0	.008, .008	.005
0.10	.025	2.0	.013, .015	.011
0.20	.025	4.0	.025, .026	.023
0.30	.025	6.0	.037, .036	.034
0.50	.025	10.0	.060, .061	.058

$$P\ (ug\ L^{-1}) = 0.0851 + 171.6754\ (\bar{abs})$$
$$r^2 = .9997$$

Dissolved Phosphorus (manual)

Sample preparation involves ultra-filtration prior to analysis using Millipore CX-10 immersible filters (MWCO 10,000). Phosphorus in the ultra-filtrate can be analyzed for both DRP and TDP (Figure 17).

Sample Preparation (ultrafiltration)

1) Assemble the apparatus (Figure 18), pass ~25 ml of DI water through the filter at $\leq$15 psi, and discard. This cleans the filter of the glycerine preservative.

2) Filter ~10 ml of sample, discard, and collect the remaining (~100 ml) ultrafiltrate.

Procedure

1) Determine total dissolved phosphorus (TDP) using the TP method (see p. 95).

2) Determine total dissolved phosphorus (TDP) using the FRP method (see p. 100).

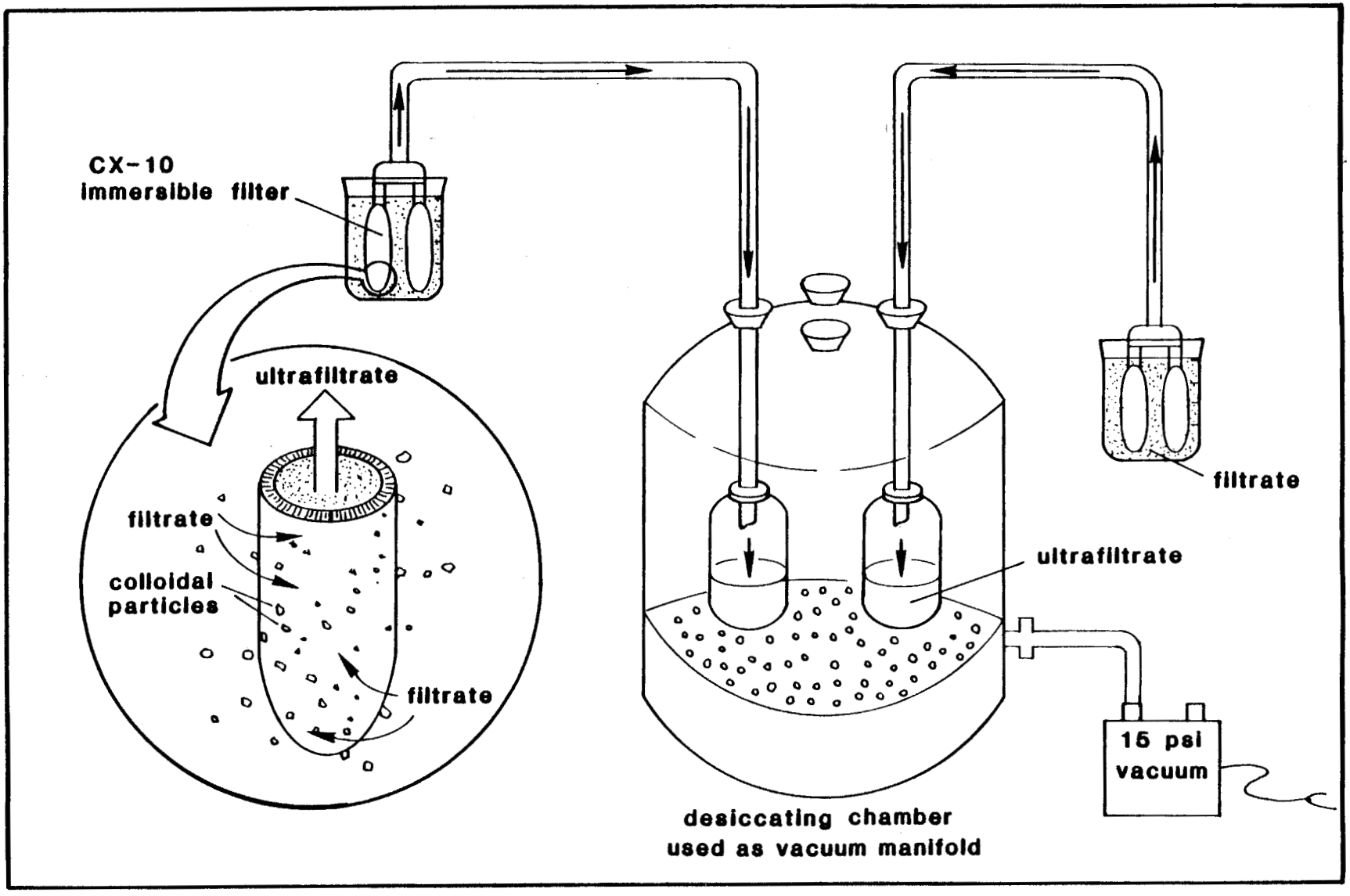

Figure 18. Apparatus used to process filtered water for ultrafiltrate (≤10,000 MWCO), showing the vacuum manifold and Millipore immersible ultra-filters, used for analysis of dissolved phosphorus fractions.

Total Kjeldahl Nitrogen and Total Phosphorus (automated)

Total Kjeldahl nitrogen (TKN) refers to a procedure for determining the organic nitrogen and total ammonia content of water. Total phosphorus (TP) and total Kjeldahl nitrogen (TKN) can be determined either simultaneously or individually using this method.

Standard Range

50-300 ug L^{-1} N; 2.5-50 ug L^{-1} P.

Upper Limit of Detection

3000 ug L^{-1} N; 100 ug L^{-1} P.

Lower Limit of Detection

Empirical: 3 ug L^{-1} N; 1 ug L^{-1} P.
Predicted: 3.5 ug L^{-1} N; 1.2 ug L^{-1} P.

Precision

7.5% at 100 ug L^{-1} N; 12% at 12.5 ug L^{-1} P.

Accuracy

$\pm$ 3% at 100 ug L^{-1} N; $\pm$ 9% at 12.5 ug L^{-1} P.

Apparatus

Technicon Autoanalyzer II equipped with 630 and 880-nm filters, 50-mm flow cells, BD 20 block digestor with 75-ml digestion tubes, and a vortex mixer.

Reagents

1) Digestion Reagent

   (a) Dissolve 2 g of mercuric oxide (HgO) into 20 ml of 20% v/v sulfuric acid (20 ml concentrated H_2SO_4 diluted to 100 ml with DI water).

   (b) Dissolve 134 g of potassium sulfate (K_2SO_4) into 500 ml of DI water, slowly add 200 ml of concentrated sulfuric acid, and cool.

   (c) Combine parts a and b, and dilute to 1 liter with DI water.

2) Sample wash - Dissolve 13.4 g of potassium sulfate (K_2SO_4) into ~500 ml of DI water containing 15 ml of concentrated

sulfuric acid, and dilute the mixture to 1 liter with DI water.

3) System wash - Add 1 ml of Triton-X wetting agent (Technicon Industries) per liter of DI water.

Total Kjeldahl reagents

4) Complexing reagent - Dissolve 1 g of sodium chloride (NaCl) and 3.2 g of sodium hydroxide (NaOH) into 500 ml of DI water, dilute to 1 liter, and add 1 ml of Brij 35 wetting agent (Technicon Industries).

5) Buffer - Dissolve 83.5 g of potassium phosphate, dibasic (K_2PHO_4), 1 g of sodium citrate-trihydrate, 20.2 g of sodium hydroxide (NaOH) and 9.3 g of Na-EDTA into 400 ml of DI water. Dilute to 500 ml with DI water, and add 0.5 ml of Brij 35.

6) Phenate

   (a) Add 25 ml of DI water to a new reagent bottle containing 500 g of crystalline phenol, and warm to dissolve by placing the bottle in hot water. Prepare this phenol stock solution as necessary.

   (b) Dissolve 2 g of potassium ferrocyanide-trihydrate, 12.5 g of sodium hydroxide (NaOH) into ~400 ml of DI water, and add 25 ml of phenol stock solution. Dilute the mixture to 500 ml with DI water, and add 0.5 ml of Brij 35.

7) Hypochlorite solution - Dilute 20 ml of Clorox (5% hypochlorite) to 100 ml with DI water, and add 0.1 ml of Brij 35.

8) Catalyst - Dissolve 0.4 g of sodium nitroferrocyanide-dihydrate into 500 ml of DI water, dilute to 1 liter, and add 1 ml of Brij 35.

Total phosphorus reagents

9) Molybdate solution - Dissolve 12 g of ammonium molybdate 4-hydate, 0.25 g of antimony potassium tartrate into ~500 ml of DI water. Add 250 ml of 20% sulfuric acid, dilute to 1 liter with DI water, and add 1 ml of Levor V wetting agent (Technicon Industries). Allow the other phosphorus reagents to pump through the autoanalyzer system for 5 minutes before introducing this solution.

10) Ascorbic acid - Dissolve 20 g of ascorbic acid into ~ 500 ml of DI water, dilute to 1 liter, and add 1 ml of Levor V.

11) Sodium chloride - Dissolve 10 g of sodium chloride (NaCl) into ~500 ml of DI water, dilute to 1 liter, and add 0.5 ml of Levor V.

Procedure

1) Pour 20 ml of unfiltered sample or standard into a block digestion tube. Reserve the same set of tubes for standards.

2) Add 2 ml of digestion reagent and ~10 teflon boiling chips.

3) Place in the block digestor and digest for 1 hour at 200 C.

4) Further digest the samples at 360 C for 20 $\pm$ 1 minutes, remove, and dilute to 20 ml with DI water while vortexing.

5) Withdraw a 4-ml aliquot using an autopipet, and add to the sampler tray.

6) Arrange the autoanalyzer (Figure 19), baseline, and calibrate the system (see Instrument Calibrations, p. 192).

NOTE: The phosphorus line should be preconditioned by pumping 1 N NaOH through the system for 5 minutes, followed by a 5 minute wash with hydrogen peroxide (20%) and then a ten minute system rinse with DI water.

7) Pump the standards and samples through the system.

Standards

Primary nitrogen standard (0.2 mg ml^{-1} N) - Dissolve 0.3824 g of ammonium chloride (NH_4Cl) into ~400 ml of DI water, and dilute to 500 ml.

Secondary nitrogen standard (2 ug ml^{-1} N) - Dilute 1 ml of the primary standard to 100 ml.

Primary phosphorus standard (0.5 mg ml^{-1} P) - Dissolve 0.1404 g of potassium phosphate, dibasic (K_2HPO_4) into ~400 ml of DI water, and dilute to 500.

Secondary phosphorus standard (0.50 ug ml^{-1} P) - Dilute 1 ml of the primary standard to 100 ml.

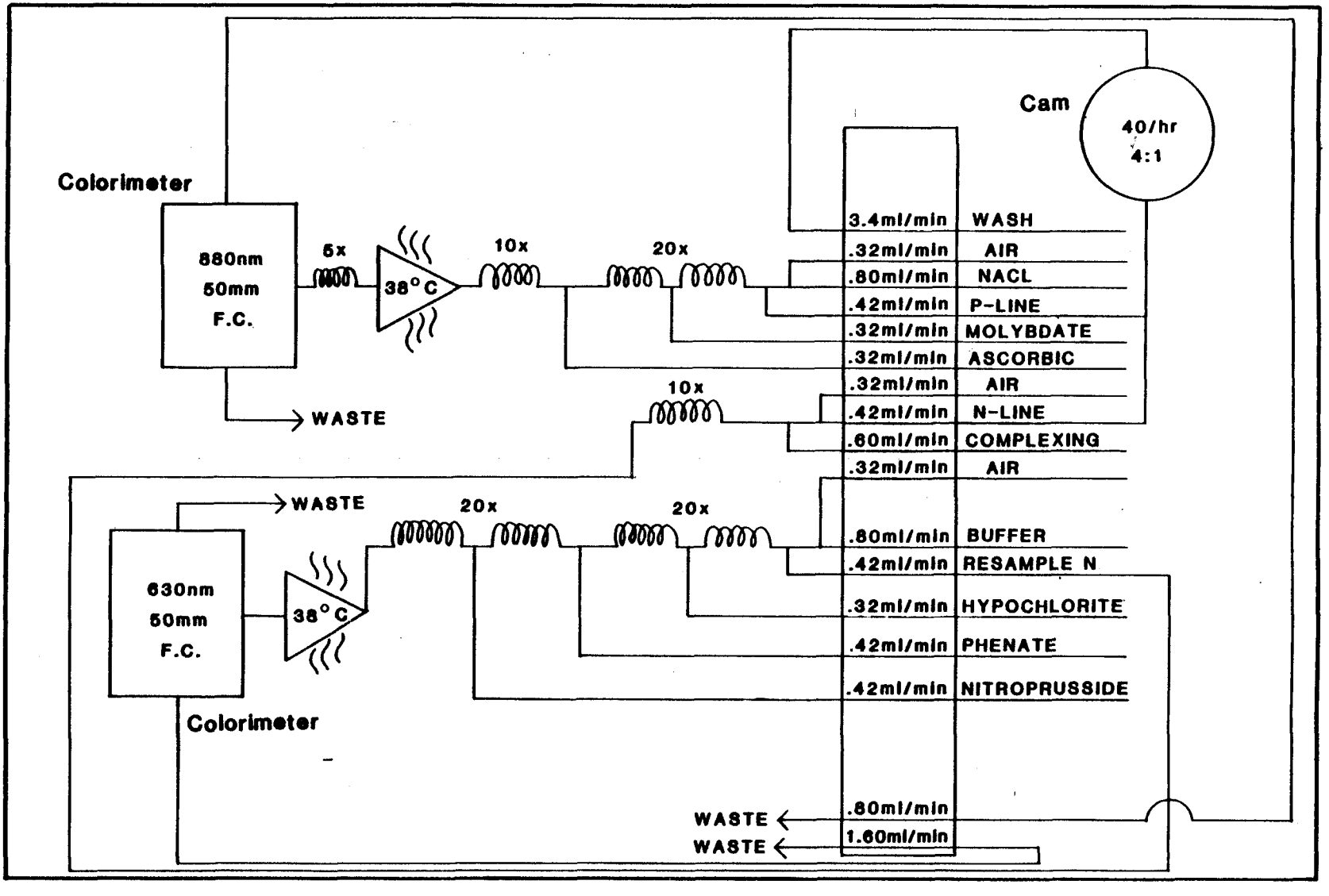

Figure 19. Autoanalyzer schematic showing reagent lines and flow rates (ml/min), cam size (number of samples per hour and sample to wash ratios), mixing coils, heating baths, and colorimeters with specified flow cells (FC) and wavelengths (nm) used in the simultaneous analysis of total phosphorus and total Kjeldahl nitrogen.

Calculations

1) Formulate separate linear equations for nitrogen and phosphorus by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample chart deflections ($\bar{\bar{cd}}$), and substitute into the appropriate regression formula (Tables 29 and 30).

Table 29. The relationship between known concentrations of nitrogen and autoanalyzer chart deflections ($\bar{\bar{cd}}$) used to calculate a standard curve in the automated analysis of total Kjeldahl nitrogen.

Secondary N standard (ml)	Total volume (L)	Nitrogen concentration (ug L^{-1} N)	Chart deflection (x)	$\bar{\bar{cd}}$ ($\bar{x}$-blank)
0.0	.020	0	4.5, 4.5	0.0
0.0	.020	0	5.0, 4.0	
0.5	.020	50	19.0, 20.0	15.0
1.0	.020	100	38.0, 36.0	32.5
1.5	.020	150	52.5, 51.5	47.5
2.0	.020	200	56.5, 67.5	62.0
3.0	.020	300	99.5,100.0	95.3

$$N\ (ug\ L^{-1}) = 0.6336 + 3.1558\ (\bar{\bar{cd}})$$
$$r^2 = .9995$$

Table 30. The relationship between known concentrations of phosphorus and autoanalyzer chart deflections ($\bar{cd}$) used to calculate a standard curve in the automated analysis of total phosphorus.

Secondary P standard (ml)	Total volume (L)	Phosphorus concentration (ug L^{-1})	Chart deflection (x)	$\bar{cd}$ ($\bar{x}$-blank)
0.0	.020	0.0	2.0, 0.0	
0.0	.020	0.0	0.0, 0.5	0.0
0.1	.020	2.5	2.5, 6.5	3.9
0.3	.020	7.5	7.5, 7.0	6.6
0.5	.020	12.5	11.5, 12.0	11.2
1.0	.020	25.0	31.0, 25.0	27.4
1.5	.020	37.5	38.0, 41.0	38.9
2.0	.020	50.0	52.0, 53.5	52.2

$$P \text{ (ug } L^{-1}) = 0.2384 + 0.9510 (\bar{cd})$$
$$r^2 = .9965$$

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PARTICULATE NUTRIENTS

The amounts of carbon (C), nitrogen (N), and phosphorus(P) contained on a 4.25 cm GF/F filter are reported as particulate nutrients. The material retained by the filter consists of detritus, bacteria, and phytoplankton; however, in oligotrophic lakes phytoplankton are considered to be primary contributors. Particulate nutrients, along with chl *a*, characterize the standing crop of primary producers. In particular, the C:N:P atom ratios are useful indicators of nutritional status with ratios greater than 106:16:1 suggesting that phosphorus limits biomass accrual (Kuenzler et al. 1979).

Filter Blanks

The amount of C, N, and P contained in a 4.25-cm GF/F filter will be reported as a particulate nutrient, and is the filter blank (FB). To avoid overestimating a particulate nutrient, the FB is subtracted from the estimated amount of carbon, nitrogen, and phosphorus. This correction is especially important when estimating the extremely low nutrient levels in oligotrophic systems.

Cleaned filters were analyzed for particulate C, N, and P to determine the mean amount (ug) $\pm$ 2 standard deviations (S.D.) per filter, and are listed below. Each FB is used in the calculation for the appropriate particulate nutrient. The corrected amount of particulate nutrient and the volume filtered (V_f) are used to calculate the in-lake concentration.

Particulate Nutrient (PN)	FB (ug + 2 S.D.)
Particulate Organic Carbon	46.2 (range 28.0-125.0)
Total Particulate Phosphorus (manual)	1.06 $\pm$ 0.24
Total Particulate Phosphorus (automated)	0.785 $\pm$ 0.264
Organic Particulate Phosphorus (manual)	1.229 $\pm$ 0.742
Inorganic Particulate Phosphorus (manual)	0.352 $\pm$ 0.348
Total Particulate Nitrogen (automated)	1.602 $\pm$ 1.108

Calculations

Particulant nutrient concentrations (ug L^{-1}) are calculated using the following general expression:

$$\text{PN concentration (ug } L^{-1}) = (PN-FB)/V_f$$

Organic Carbon (manual)

Measurements of particulate organic carbon provide a basis for estimating the amount and the energy content of organic material. This procedure measures reduced carbon by wet oxidation using acid dichromate, but is referenced to carbohydrate carbon. This technique has been criticized because of an apparent inefficiency in oxidizing nitrogen-rich compounds. However, Newel (1982) has shown that these compounds are oxidized using dichromate. Thus, realistic estimates of the total organic-carbon content are possible.

As carbon is oxidized by the dichromate mixture, the color of the oxidant changes from yellow to green. The decrease in the absorbance at 440 nm is linear with increasing carbon concentration.

Standard Range

60-600 ug as C.

Upper Limit of Detection

600 ug as C.

Lower Limit of Detection

Empirical: 30 ug C.
Predicted: 24 ug C.

Precision

25% at 300 ug C.

Accuracy

± 9% at 300 ug C.

Apparatus

Spectrophotometer (400 nm), drying oven (100 C), centrifuge, 15-ml glass centrifuge tubes, 50-ml Pyrex erlenmeyer flasks, and 10-mm cuvettes.

Reagents

1) Acid dichromate oxidant - Dissolve 4.84 g of potassium dichromate ($K_2Cr_2O_7$) in 20 ml of DI water. Slowly add the dichromate solution to 500 ml of concentrated sulfuric acid, cool, and dilute to 1 liter with concentrated sulfuric acid.

2) Phosphoric acid - Concentrated reagent-grade phosphoric acid (H_3PO_4).

NOTE: Use glass pipets and Pyrex containers for preparing and storing all reagents.

Procedure

1) Place the particulate C filter, seston side down, into a 50-ml erlenmeyer flask.

2) Add 1.8 ml of DI water, 1 ml of phosphoric acid, and swirl to mix.

3) Add 2 ml of the oxidant, swirl to mix, cover with aluminum foil, and place in the drying oven at 100 C for 30 minutes. Swirl the flask and continue heating for an additional 30 minutes.

4) Cool, add 45 ml of DI water, and vigorously mix using a Teflon rod.

5) Allow the filter slurry to settle, decant 10 ml of the solution into a 15-ml centrifuge tube, and centrifuge for 15-20 minutes at 2500 RPM.

6) Measure the absorbance at 440 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary dextrose standard (30 mg ml^{-1} C) - Dissolve 18.75 g of dextrose-anhydrous into 200 ml of DI water, and dilute to 250 ml.

Secondary standard (600 ug ml^{-1} as C) - Dilute 2 ml of the primary standard to 100 ml.

Calculations

As absorbance at 440 nm decreases with an increasing carbon content, absorbances of the standards and samples must be subtracted from the blank.

1) Formulate a linear equation by regressing concentrations (Y-axis) against averaged absorbances (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate concentrations by subtracting sample absorbances from the blank value and substitute into the regression formula.

3) Subtract the FB value, and divide by the volume filtered (Table 31).

Table 31. The relationship between known concentrations of carbon and absorbances ($\bar{\bar{abs}}$) at 440 nm used to calculate a standard curve in the analysis of particulate organic carbon.

Secondary standard (ml)	Total volume (L)	Carbon (ug)	Absorbance (x)	$\bar{\bar{abs}}$ (blank-$\bar{x}$)
0.00	1.8	0	.214, .218	.000
0.10	1.8	60	.211, .212	.004
0.20	1.8	120	.190, .190	.026
0.30	1.8	180	.182, .182	.034
0.50	1.8	300	.125, .136	.085
1.00	1.8	600	.037, .034	.180

$$C\ (ug) = 37.0958 + 3153.2679\ (\bar{\bar{abs}})$$
$$r^2 = .9881$$

Phosphorus (manual)

Total particulate phosphorus (TPP) estimates the phosphorus content of the limnetic phytoplankton, bacteria, and detritus. In practice, TPP from oligotrophic lakes is considered to represent phosphorus within the phytoplankton; and is determined as reactive phosphorus after sulfuric acid-persulfate digestion. TPP can be indirectly determined by difference (TP-TFP) (Figure 17); however, the accuracy of direct analysis is preferred given the extremely low phosphorus levels within oligotrophic systems.

Standard Range

1-14 ug P.

Lower Limit of Detection

Empirical: 0.05 ug P.
Predicted: 0.02 ug P.

Precision

3% at 4.5 ug P.

Accuracy

±3% at 4.5 ug P.

Apparatus

Spectrophotometer (882 nm), autoclave (121 C, 15 psi) centrifuge, 50-ml erlenmeyer flasks, 15-ml disposable centrifuge tubes, and 10-mm cuvettes.

Reagents

1) Digestion acid (3.6 N H_2SO_4) - Slowly add 50 ml of concentrated sulfuric acid[2] to 400 ml of DI water, cool, and dilute to 500 ml.

2) Digestion solution - Dissolve 9.99 g of potassium persulfate ($K_2S_2O_8$) into 250 ml of DI water. Add 16.7 ml of digestion acid, and dilute to 1 liter with DI water.

3) Mixed reagent I (MR I) - See TP/TFP reagents (p. 98).

Procedure

1) Place the particulate nutrient filter into a 50-ml erlenmeyer flask seston side up.

2) Add 30 ml of digestion solution, swirl to mix, and cover with a 15-ml polypropylene beaker.

3) Autoclave (121 C, 15 psi) for 30 minutes, cool, and swirl to mix.

4) Withdraw a 5-ml aliquot using an autopipet, place into a 15-ml centrifuge tube, add 1 ml of MR I, and invert to mix.

5) Centrifuge at 2500 rpm for 20 minutes during which time color development is completed.

6) Measure the absorbance at 882 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary standard (0.45 mg ml^{-1} P) - Dissolve 1.2640 g of potassium phosphate-dibasic (K_2HPO_4) in 500 ml of DI water, and dilute to 500 ml.

Secondary standard (4.5 ug ml^{-1} P) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentrations (Y-axis) against the averaged absorbances minus the blank (X-axis), and calculate the coefficient of determination (r^2).

2) Determine sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the regression formula (Table 32).

3) Subtract the filter blank (FB) value, and divide by the volume of lake water (liters) filtered.

Table 32. The relationship between known amounts of phosphorus and absorbances ($\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}}$) at 882 nm used to calculate a standard curve in the analysis of total particulate phosphorus.

Secondary standard (ml)	Total volume (L)	Phosphorus (ug)	Absorbance (x)	$\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}}$ ($\bar{x}$-blank)
0.0	.030	0.0	.005, .005	.000
0.3	.030	1.35	.033, .034	.029
0.5	.030	2.25	.051, .055	.048
1.0	.030	4.5	.102, .104	.098
2.0	.030	9.0	.200, .202	.196
3.0	.030	13.5	.295, .300	.293

$$P\ (ug) = 0.0114 + 45.9812\ (\bar{\bar{a}}\bar{\bar{b}}\bar{\bar{s}})$$
$$r^2 = 1.0000$$

Inorganic and Organic Phosphorus (manual)

Total particulate phosphorus (TPP) is comprised of both inorganic (IPP) and organic (OPP) phosphorus. The proportion of each varies according to lake type; i.e., glacial, organically stained, or clear. For example, IPP (rock phosphorus) has been found to dominate TPP in glacial lakes (Edmundson and Koenings 1985). Both IPP and OPP are determined on the same filter with acidified ammonium fluoride used to first extract IPP (Figure 20), and then OPP being determined after persulfate digestion.

Standard Range

0.675-6.75 ug P (IPP), 1.35-13.5 ug P (OPP).

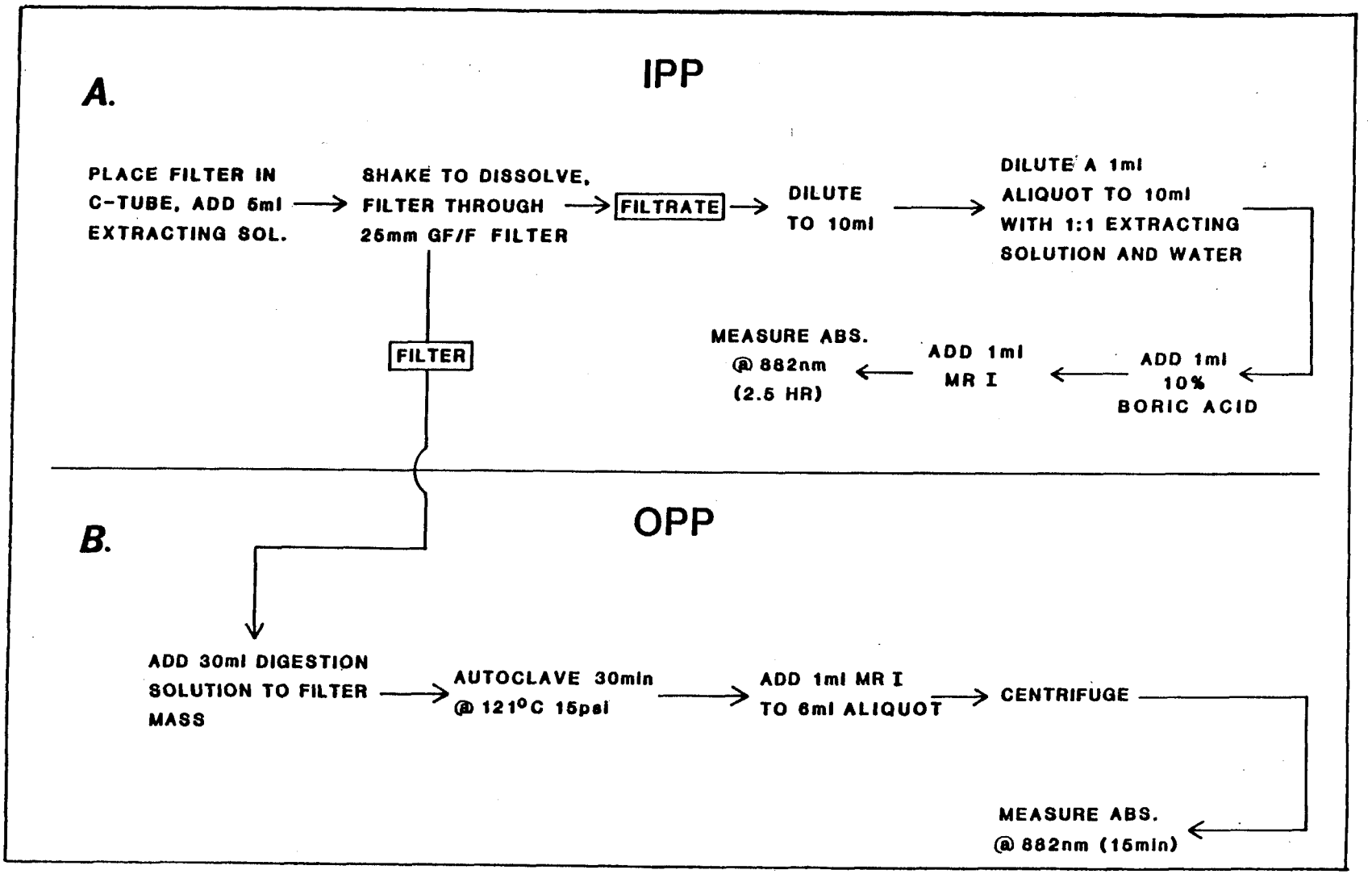

Figure 20. Flow diagram of the analysis procedures for determining (A) inorganic particulate phosphorus (IPP) using fluoride extraction, and (B) organic particulate phosphorus (OPP) after acid digestion.

Lower Limit of Detection

Empirical: 0.05 ug P (IPP), 0.05 ug P (OPP).
Predicted: 0.08 ug P (IPP), 0.03 ug P (OPP).

Precision

12% at 2.25 ug P (IPP), 10% at 4.5 ug P (OPP).

Accuracy

± 3% at 2.25 ug P (IPP), ± 4% at 4.5 ug P (OPP).

Apparatus

Spectrophotometer (882 nm), autoclave (121 C, 15 psi), 25-mm filter tower, centrifuge, 50-ml erlenmeyer flasks, 15-ml centrifuge tubes, and 10-mm cuvettes.

Reagents

1) 1 N Ammonium fluoride - Dissolve 37 g of NH_4F into 500 ml of DI water, and dilute to 1 liter.

2) 0.5 N Hydrochloric acid - Add 20.8 ml of concentrated HCl to 400 ml of DI water, cool, and dilute to 500 ml.

3) Extracting solution - Combine 15 ml of 1 N NH_4F, 100 ml of 0.5 N HCl, and 385 ml of DI water.

4) Ammonium molybdate solution - Dissolve 15 g of ammonium molybdate-4-hydrate into 400 ml of DI water, and dilute to 500 ml.

5) Sulfuric acid solution - Slowly add 70 ml of concentrated H_2SO_4 to 450 ml of DI water.

6) Ascorbic acid solution - Dissolve 5.4 g of ascorbic acid in 100 ml of DI water. Prepare fresh daily.

7) 10% Boric acid solution - Add 10 g of boric acid to 100 ml of DI water, and filter the mixture through a 'fast' pleated filter.

8) Potassium antimony-tartrate solution - Dissolve 0.34 g of antimony potassium-tartrate-trihydrate into 200 ml of DI water, and dilute to 250 ml.

9) Digestion solution - Dissolve 9.99 g of potassium persulfate ($K_2S_2O_8$) into 250 ml DI water, add 16.7 ml of digestion acid, and dilute the mixture to 500 ml.

10) Special mixed reagent (SR) - Combine in order, 20 ml of ammonium molybdate solution, 50 ml of sulfuric acid solution, 20 ml of ascorbic acid solution, and 10 ml of the potassium antimony-tartrate solution.

11) Mixed reagent I (MR I) - See TP/TFP reagents 1, 2, and 5 (p. 98).

Procedure

1) Place a particulate nutrient filter into a disposable 15-ml centrifuge tube.

2) Add 5 ml of the extracting solution, cap, and shake vigorously until the contents form a slurry (~1 minute).

3) Pour the slurry into a 25-mm filter tower, rinse the tube with ~2 ml of DI water, and filter the mixture through a Whatman 2.5-cm GF/F glass-fiber filter.

4) Collect the extract (IPP) in a clean centrifuge tube, dilute to 10 ml with DI water; and place the filter mass into a 50-ml erlenmyer flask.

   NOTE: Dilutions are required for extracts from glacial waters; use a 1:1 mixture of extracting solution and DI water as the diluent prior to step 5.

5) Add 1 ml of 10% boric acid solution to the IPP extract and invert the centrifuge tube to mix.

6) Add 1 ml of SR, invert to mix, and allow 20 minutes for color development.

7) Follow steps 2-5 of the TPP procedure (p. 116) for determining the OPP held in the filter mass.

8) Measure the absorbances at 882 nm against a DI water blank (see Instrument Calibrations, p. 192).

Standards

Primary standard (0.45 mg ml^{-1} P) - Dissolve 0.1264 g of potassium phosphate dibasic (K_2HPO_4) into 400 ml of DI water, and dilute to 500 ml.

IPP secondary standard (2.25 ug ml^{-1} P) - Dilute 0.5 ml of the primary standard to 100 ml.

OPP secondary standard (4.5 ug ml^{-1} P) - Dilute 1 ml of the primary standard to 100 ml.

Calculations

1) Formulate separate linear equations by regressing concentrations (Y-axis) against averaged absorbances minus the blank (X-axis), and calculate the coefficients of determination (r^2) (Tables 33 and 34).

2) Calculate sample concentrations by subtracting the blank value from the sample absorbances, and substitute into the appropriate regression formula.

3) Subtract the appropriate FB values, divide by the volume (liters) of the lake water filtered and, if necessary, multiply by the dilution factor (p. 39).

Table 33. The relationship between known amounts of phosphorus and absorbances ($\bar{\bar{\bar{abs}}}$) at 882 nm used to calculate a standard curve in the analysis of inorganic particulate phosphorus (IPP).

Secondary standard (ml)	Total volume (L)	Phosphorus (ug)	Absorbance (x)	$\bar{\bar{\bar{abs}}}$ ($\bar{x}$-blank)
0.0	.010	0.00	.001, .001	.000
0.3	.010	0.68	.042, .043	.042
0.5	.010	1.13	.069, .072	.070
1.0	.010	2.25	.143, .144	.143
2.0	.010	4.50	.284, .288	.285
3.0	.010	6.75	.435, .422	.428

$$IPP\ (ug) = 0_20117 + 15.7434\ (\bar{\bar{\bar{abs}}})$$
$$r^2 = 1.0000$$

Table 34. The relationship between known amounts of phosphorus and absorbances ($\bar{\bar{abs}}$) at 882 nm used to calculate a standard curve in the analysis of organic particulate phosphorus (OPP).

Secondary standard (ml)	Total volume (L)	Phosphorus (ug)	Absorbance (x)	$\bar{\bar{abs}}$ ($\bar{x}$-blank)
0.0	.030	0.00	.000, .000	.000
0.3	.030	1.35	.025, .025	.025
0.5	.030	2.25	.042, .043	.043
1.0	.030	4.50	.083, .083	.083
2.0	.030	9.00	.170, .182	.176
3.0	.030	13.50	.254, .269	.262

$$OPP\ (ug) = 0_20709 + 51.2305\ (\bar{\bar{abs}})$$
$$r^2 = .9997$$

Nitrogen and Phosphorus (automated)

Particulate nitrogen, along with carbon and phosphorus, is a primary nutrient of the plankton. After phosphorus, lack of nitrogen is most likely to be limiting to primary production. Measurements of these two nutrients, along with carbon, allow for the calculation of ratios which are useful indicators of the nutritional status of the plankton.

This method is based upon the simultaneous determination of total Kjeldahl nitrogen and total phosphorus (p. 104).

Standard Range

2-60 ug N, 1-12 ug P.

Lower Limit of Detection

Empirical: 1 ug N, 0.6 ug P.
Predicted: 0.1 ug N, 0.4 ug P.

Precision

4% at 40.0 ug N, 6% at 4.5 ug P.

Accuracy

±3% at 40.0 ug N, ±7% at 4.5 ug P.

Apparatus

Technicon Autoanalyzer II equipped with 630- and 880-nm filters and 50-mm flow cells, block digestor, vortex mixer, centrifuge, 15-ml disposable centrifuge tubes with caps, and 75-ml digestion tubes.

Reagents

1) Prepare reagents 1-11 as described in the TKN/TP methods (p. 104).

2) Acid diluent - Add 0.5 ml Levor V wetting agent (Technicon Industries) per liter of DI water.

Procedure

1) Loosely fold the particulate nutrient filter with the seston inside, place into a digestion tube, and add 20 ml of DI water covering the filter.

2) Follow steps 2-4 of the TKN/TP procedure (p. 106).

3) Withdraw an 8-ml aliquot using an autopipet, place into a 15 ml disposable centrifuge tube, and cap.

4) Centrifuge at 2500 rpm for 15 minutes.

5) Pour ~4 ml of sample or standard into the sampler tray.

7) Arrange the autoanalyzer (Figure 21), baseline, calibrate (see Instrument Calibrations, p. 192), and pump the standards and samples through the system.

Standards

Primary nitrogen standard (2 mg ml^{-1} N) - Dissolve 5.3619 g of glycine into ~450 ml of DI water, and dilute to 500 ml.

Secondary nitrogen standard (20 ug ml^{-1} N) - Dilute 1 ml of the primary standard to 100 ml.

Primary phosphorus standard (0.45 mg ml^{-1} P) - Dissolve 1.2640 g of potassium phosphate dibasic (K_2PHO_4) into 400 ml of DI water, and dilute to 500 ml.

Secondary phosphorus standard (22.5 ug ml^{-1} P) - Dilute 5 ml of the primary standard to 100 ml.

Calculations

1) Formulate separate linear equations by regressing concentrations (Y-axis) against the averaged chart

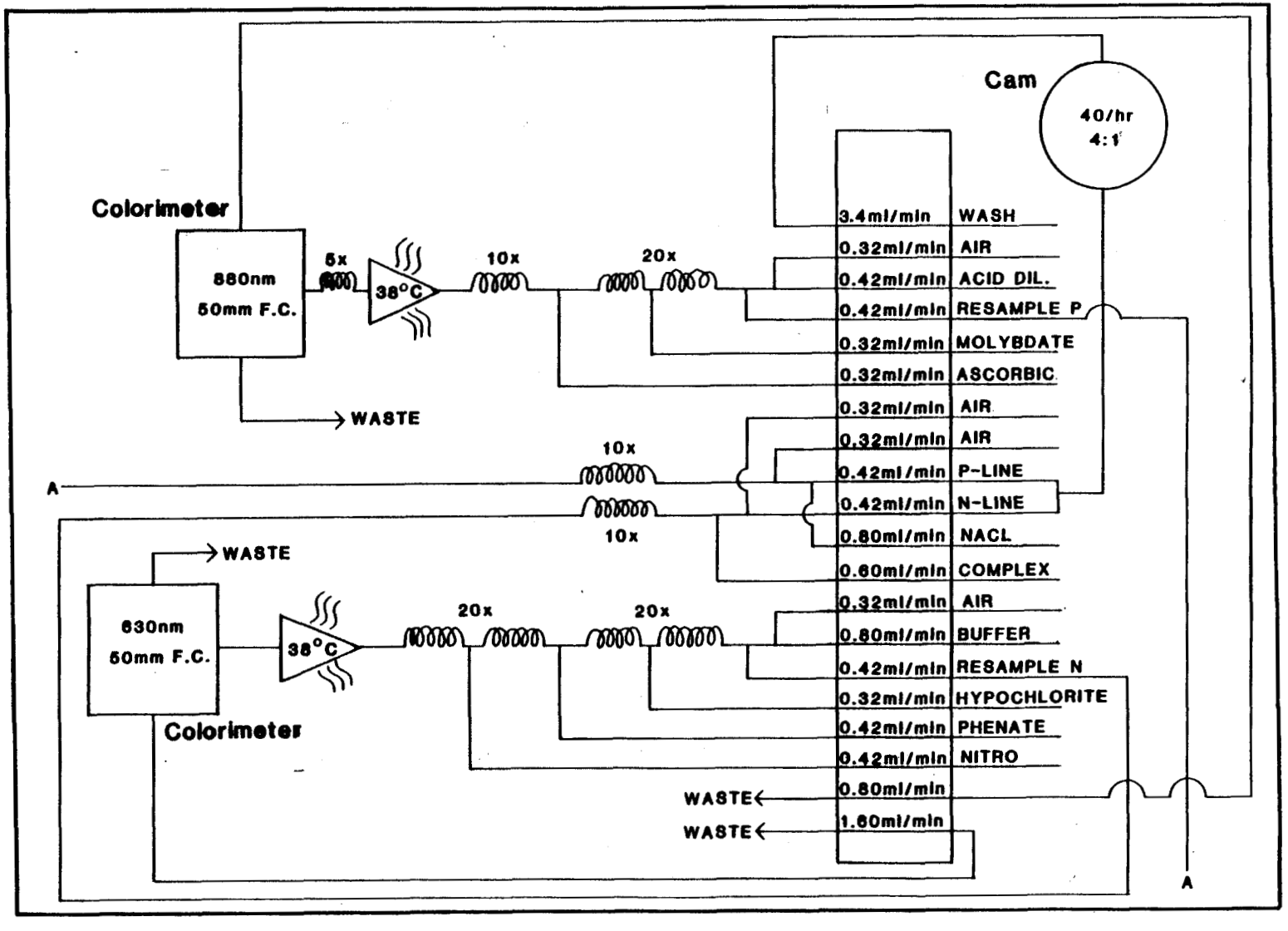

Figure 21. Autoanalyzer schematic showing reagent lines and flow rates (ml/min), cam size (number of samples per hour and sample to wash ratio), mixing coils, heating baths, and colorimeters with specified flow cells (FC) and wavelength (nm) used in the simultaneous analysis of particulate nitrogen and phosphorus.

deflections minus the blank (X-axis), and calculate coefficients of determination (r^2).

2) Calculate sample concentrations by subtracting the appropriate blank value from the sample chart deflections, and substitute into the appropriate regression formula (Tables 35 and 36).

3) Subtract the appropriate FB value, and divide by the volume (liters) of lake water filtered.

Table 35. The relationship between known amounts of nitrogen and autoanalyzer chart deflections ($\bar{\bar{cd}}$) used to calculate a standard curve in the automated analysis of particulate nitrogen.

Secondary N standard (ml)	Total volume (L)	Nitrogen (ug N)	Chart deflection (x)	$\bar{\bar{cd}}$ ($\bar{x}$-blank)
0.0	.020	0	1, 1	0.0
0.1	.020	2	6, 6	5.0
0.2	.020	4	11, 11	10.0
0.3	.020	6	16, 16	15.0
0.5	.020	10	26, 26	25.0
1.0	.020	20	50, 51	49.5
2.0	.020	40	100, 101	99.5

$$N\ (ug) = -0.0145 + 0.4025\ (\bar{\bar{cd}})$$
$$r^2 = 1.0000$$

Table 36. The relationship between known amounts of phosphorus and autoanalyzer chart deflections ($\bar{\bar{cd}}$) used to calculate a standard curve in the automated analysis of total particulate phosphorus.

Secondary P standard (ml)	Total volume (L)	Phosphorus (ug)	Chart deflection (x)	$\bar{\bar{cd}}$ ($\bar{x}$-blank)
0.00	.020	0.00	0, 0	0.0
0.05	.020	1.13	8.5, 8	8.8
0.10	.020	2.25	17, 17	17.0
0.20	.020	4.50	40, 38	39.0
0.30	.020	6.75	64, 59	61.5
0.40	.020	9.00	87, 80	83.5
0.50	.020	11.25	105, 107	106.0

$$P\ (ug) = 0_{2}2537 + 0.1048\ (\bar{\bar{cd}})$$
$$r^2 = .9985$$

Phosphorus and Nitrogen in Fish Tissue

Nitrogen (N) and phosphorus (P) levels in sockeye (Oncorhynchus nerka) and coho (O. kitsutch) salmon are used to estimate contributions of decomposing carcasses to in-lake nutrient levels. In addition, rearing juveniles and smolts are also examined to obtain both the N and P levels within the fish community, and the quantity of nutrients leaving the lake, respectively. Either whole-fish or fish-core samples are used to determine the N and P content of fish tissue. The N and P in fish tissue is determined after sulfuric acid digestion using the TP/TKN method for water samples.

Apparatus

Technicon Autoanalyzer II equipped within 630- and 880-nm filters BD-40 block digestor, 75-ml digestion tubes, autoclave (121 C, 15 psi), autoclavable plastic bags, mechanical grinder, and blender.

Reagents

Prepare reagents as described in the particulate nitrogen and phosphorus method (p. 122).

Standards

Identical standards were used for whole and cored samples.

1) Primary nitrogen standard (2 mg ml^{-1} P) - Dissolve 2.6831 g of glycine into ~200 ml of DI water, and dilute to 250 ml.

2) Secondary standard (20 ug ml^{-1} N) - Dilute 1 ml of the primary N standard to 100 ml.

3) Primary phosphorus standard (0.45 mg ml^{-1} P) - Dissolve 0.6322 g of potassium phosphate-dibasic into ~200 ml of DI water, and dilute to 250 ml.

4) Secondary standard (22.5 ug ml^{-1} P) - Dilute 5 ml of primary P standard to 100 ml.

Prepare standard curves with ranges of 10-3000 ug L^{-1} N, and 50-450 ug L^{-1} P.

A. Whole Carcass Analysis

Procedure

1) Grind the carcass using a mechanical grinder, add to two autoclavable bags, and add 500 ml of DI water to each bag.

2) Autoclave (121 C, 15 psi) for 30 minutes, cool, and measure the combined volume of slurry.

3) Remove two 100-ml subsamples of slurry, place into 125-ml polybottles, and freeze for later analysis.

4) Thaw, shake the sample well, withdraw a 5-ml aliquot using an autopipet, place into a beaker, and add 45 ml of DI water.

5) While stirring withdraw a 1-ml aliquot, place into a digestion tube; add 20 ml DI water, 2 ml of digestion reagent, and 10 teflon boiling chips.

6) Digest in the block digestor at 200 C for 1 hour. Include several DI water blanks to be used as a later diluent.

7) Digest for an additional 20 $\pm$1 minutes at 360 C, and dilute the residue with 20 ml of DI water.

8) Withdraw a 0.05-ml aliquot, place in the sampler tray, and add 3 ml of digested blank water.

9) Arrange the autoanalyzer (Figure 21), baseline, calibrate (see Instrument Calibrations, p. 192), and pump the standards and samples through the systems.

Calculations

1) Formulate separate linear equations by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficients of determination (r^2).

2) Calculate sample concentrations by subtracting the appropriate blank value from the sample chart deflections and substitute into the appropriate regression formula (e.g., Tables 29 and 30).

3) To estimate the N or P concentration in the slurry use the following:

   N or P ug per ml of fish slurry = N or P (ug L^{-1}) x 16.2.

4) Multiply by the total volume (ml) of fish slurry (see procedure, step 2) to obtain the amount of N and P in the fish carcass.

5) Divide N by 14 and P by 31 and calculate an N:P atom ratio (Table 37).

B. Cored Sample Analysis

Procedure

1) Remove several (~6) 1.25-cm diameter cores along the mid-line of the weighed fish, combine the cores, and weigh.

2) Place the cores into an autoclavable bag, add ~100 ml of DI water, and autoclave (121 C, 15 psi) for 30 minutes.

3) Pour the slurry into a blender, dilute to 1 liter with DI water, and homogenize at high speed.

4) Remove two 100-ml subsamples of slurry, place into 125-ml polybottles, and freeze for later analysis.

5) Thaw, shake the sample, withdraw a 1-ml aliquot using an autopipet, and place into a digestion tube.

6) Add 20 ml of DI water, 2 ml of digestion reagent, and 10 teflon boiling chips.

7) Follow the whole carcass procedure, steps 6 and 7.

8) Withdraw a 0.2-ml aliquot, place in the sampler tray, and dilute to 4 ml with digested blank water.

Table 37. Ammounts of nitrogen and phosphorus, and the nitrogen to phosphorus atom ratios in (A) sockeye juveniles (fall fry) and (B) smolts from Alaskan lakes during 1983.

System	Life stage (sample size)	Phosphorus (mg) Total	Reactive	Percent wet weight	Nitrogen (mg) Ammonium	Nitrite + Nitrate	TKN*	Percent wet weight	TKN:TP (by atoms)
Redoubt	A (6)	2.0	1.3	0.309	0.0	0.0	20.0	3.02	22:1
Hidden	A (15)	1.0	0.6	0.322	0.0	0.0	7.0	2.37	17:1
Larson	A (18)	0.5	0.3	0.210	0.0	0.0	6.0	2.72	27:1
McDonald	A (13)	6.0	1.7	0.425	0.0	0.0	28.0	2.00	22:1
Tustumena	B (24)	4.1	2.0	0.243	0.0	0.0	32.2	1.89	17:1
Falls	B (19)	5.1	2.8	0.302	0.0	0.0	28.5	1.67	12:1
Larson	B (19)	9.8	3.7	0.038	0.0	0.0	64.2	2.14	15:1
Leisure	B (10)	4.5	3.2	0.250	0.0	0.0	47.5	2.64	23:1

*Total Kjeldahl nitrogen

9) Arrange the autoanalyzer (Figure 21), baseline, calibrate (see Instrument Calibrations, p. 192), and pump the standards and samples through the system.

Calculations

1) Formulate separate linear equations by regressing concentrations (Y-axis) against averaged chart deflections minus the blank (X-axis), and calculate the coefficients of determination (r^2).

2) Calculate sample concentrations by subtracting the appropriate blank value from the sample chart deflections, and substitute into the appropriate regression formula (e.g., Tables 29 and 30).

3) To estimate the amount of N or P (ug) of core sample use the following:

$$\text{N or P (ug) per g of core} = \frac{\text{N or P (ug L}^{-1}\text{)} \times 400.}{\text{core weight}}$$

4) Multiply by the weight (g) of the whole fish to obtain the amount of N and P in the fish carcass.

5) Divide N by 14 and P by 31 and calculate a N:P atom ratio (Table 37).

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PRIMARY PRODUCTIVITY

Primary productivity is the rate of biomass accrual by autotrophic organisms. These organisms include limnetic algae and bacteria as well as littoral macrophytes and periphyton. A majority of sockeye salmon nursery-lakes have littoral areas or volumes that are extremely limited emphasizing the importance of limnetic primary production. In the limnetic zone of oligotrophic lakes, photosynthetic phytoplankton are responsible for a majority of primary productivity; but rates are low requiring a precise method of measurement. Thus, estimating primary production through small increases in oxygen (O_2) is replaced by determining the amount of carbon (CO_2) used during photosynthesis:

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{temp.}]{\text{light}} C_6H_{12}O_6 + 6\ O_2 .$$

Measurements of primary productivity are made using carbon-14 (C-14) radioisotopes. Tracer amounts of C-14, added to lake samples, are assimilated by algal cells over a known time that the sample is incubated in situ. Following incubation, the samples are filtered and the amount of C-14 on the filter is determined using liquid scintillation spectrometry. Using these results, day-rate estimates of carbon assimilation (mg C/m^3/day) are made at different depths, and when integrated through the euphotic zone are used to estimate areal productivity (mg C/m^2/day).

Carbon-14 Assimilation

A. Field Methods

Apparatus

Van Dorn sampler, underwater photometer, temperature probe, pH meter, vacuum pump, 0.25-ml autopipet, 125-ml BOD bottles, plexiglass BOD bottle holders, 300-ml filter tower, 100-ml graduate cylinder, and 20-ml polyethylene scintillation vials.

Reagents

1) Carbon-14 isotope - 20.9 u Curies (uCi)/ml as sodium bicarbonate, C-14.

2) Lugol's acetate solution - See Preservatives for Sample Storage, (p. 15).

3) 37% Formaldehyde.

Procedure

1) Determine the 1% PAR compensation point (see Photosynthetically Active Radiation p. 22).

2) Collect water using an opaque sampler from three depths per station: 1 m, mid-euphotic, and compensation depth. Measure the pH and, if a temperature profile is not taken, record the temperature of each sample.

3) Add 100 ml of lake water to four 125-ml BOD incubation bottles for each depth sampled with two of the bottles taped and/or painted to prevent light penetration (Table 38).

NOTE: Use of two dark and two light bottles provides for replication of each treatment. However, one of the dark bottles can serve as a 'killed control' by the addition of formaldehyde and is used to separate bacterial uptake from inorganic adsorption.

Table 38. Carbon-14 incubation bottles used for in-situ radiocarbon experiments and the reactions occurring within each treatment.

Bottle type	Sample volume (ml)	Formaldehyde (37%) (ml)	Reactions
Light	100	0	Algal + inorganic + bacterial C-14 uptake
Dark	100	0	Inorganic + bacterial C-14 uptake
Kill	95	5	Inorganic adsorption C-14 uptake

4) Add 0.25 ml (5.2 u Ci) of C-14 isotope ($NaHC^{14}O_3$) to each incubation bottle using an automatic pipet.

5) Attach the bottles into a notched plexiglass plate (Figure 22A) or similar device so that the light bottles are unobstructed. Suspend each plate (Figure 22B) at the depth from which the samples were collected, and record the time (T_o).

6) After ~4 hr retrieve the samples, add 1 ml of Lugol's acetate solution, invert to mix, and record the time (T_f). If day rate estimates are necessary, see p. 140.

7) Invert each bottle several times, and filter the contents through separate Whatman 2.4-cm GF/F filters at 15 psi.

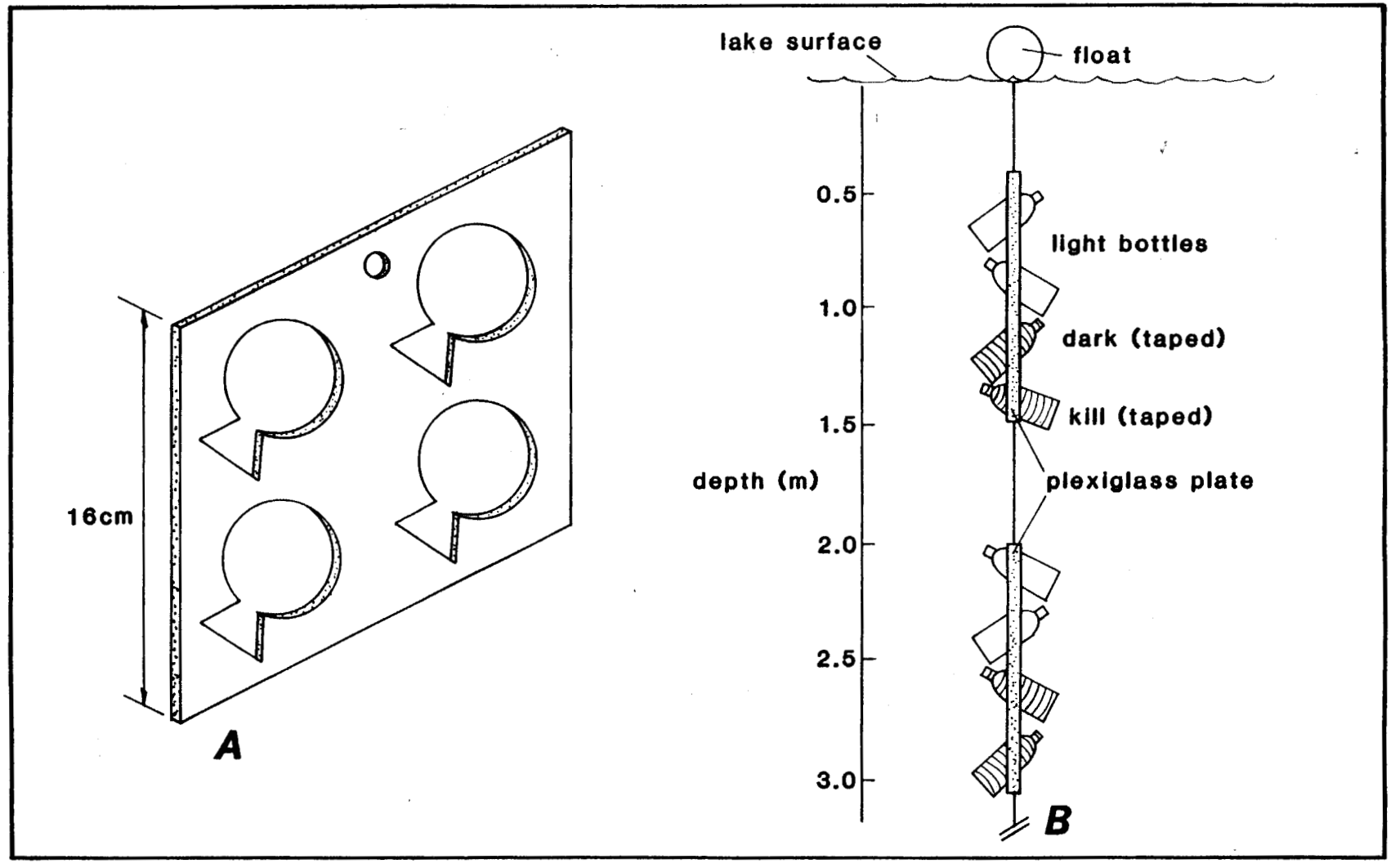

Figure 22. Plexiglass plate (A) with lock and key cut-outs used to fasten incubation bottles and (B) arrangement of a series of plates in the water column used in estimating carbon assimilation rates.

Place each filter into a labeled polyethylene scintillation vial and store frozen.

NOTE: Label only the cap of the scintillation vial and avoid using tape.

8) Soak and scrub the incubation bottles, rinse several times with tap water, DI water, and allow to drip dry.

B. Laboratory Methods

The amount of carbon-14 contained on a filter is quantified using liquid scintillation spectrometry. As C-14 atoms disintegrate they emit energetic electrons known as beta particles. When these beta particles interact with a scintillation cocktail photons are emitted (disintegrations), a portion of which are counted by the sensitive photomultiplier tubes of the spectrometer.

Apparatus

Liquid scintillation spectrometer and 10-ml dispensette.

Reagents

1) Aquasol II scintillation cocktail - New England Nuclear, Boston, MA.

2) 0.5 N Hydrochloric acid - Slowly add 32 ml of concentrated HCl into ~750 ml of DI water, and dilute to 1 liter.

Quench Curves and Counting Efficiency

Since the liquid scintillation spectrometer does not detect all the disintegrations of a sample, the relationship between sample counts (CPM) and actual disintegrations (DPM) must be established. The ratio of CPM versus DPM is the counting efficiency (E).

$$E = \frac{CPM}{DPM} \times 100$$

However, counting efficiency is affected by quenching. Quenching decreases the ability (efficiency) of the spectrometer to detect beta particles, and is caused by a number of chemical or optical effects such as sample composition, color, vial type, or filter orientation. If quenching is constant, counting efficiency remains constant, but if quenching varies either the counting efficiency of each sample must be determined or a quench curve derived to cover

the range of quenching. The varying yellow color of lake water and Lugol's acetate solution are primary quenching agents. We derived a quench curve utilizing the sample channels ratio (SCR) method described by Baille (1959), Herberg (1960), Bruno and Christian (1962), and Bush (1976). A series of vials containing varying amounts of Lugol's acetate solution and equal amounts of carbon-14 (DPM) were counted at two different energy levels [e.g., red (R) and green (G) channels]. A linear relationship was derived from the ratio of the CPM in the two channels (SCR) and counting efficiency (E) (Table 39).

Procedure

1) Thaw the filters and place seston side up in scintillation vials.

2) Add 0.3 ml of 0.5 N HCl to saturate the filters and purge inorganic $^{14}CO_2$ while in the fume hood (~2 hours).

3) Add 10 ml of scintillation cocktail (Aquasol II), number the cap, and load the vials into the liquid scintillation spectrometer.

4) Adjust the instrument to the settings listed below:

   a) Depress the adjustable discriminator.
   b) Red channel gain to 6.5%.
   c) Red channel upper limit to 1000.
   d) Red channel lower limit to 30.
   e) Green channel gain to 6.5%.
   f) Green channel upper limit to 1000.
   g) Green channel lower limit to 300.
   h) Cycle mode to 2.
   i) Number of counts to X1.
   j) Upper counting limit to 20,000.
   k) Counting time at 10 minutes. (If the counts are <1500 increase the counting time to 20 minutes.)

5) Depress 'load' and then 'operate'. The spectrometer will automatically load, count, and following completion of the last sample, terminate the counting sequence.

NOTE: Record the discrimination settings used for each data set on the actual printout.

Calculations

1) Formulate a linear equation by regressing E (Y-axis) against SCR (X-axis), and calculate the coefficient of determination (r^2).

2) Determine sample counting efficiencies by substituting sample SCR values into the appropriate regression formula (Table 39).

3) Divide sample counts from the red channel by E to derive corrected disintegrations per minute (DPM).

Table 39. CPM from red (R) and green (G) channels used to determine sample channels ratio (SCR), and CPM (R) and DPM used to calculate counting efficiencies (E).

DPM	R (CPM)	G (CPM)	SCR	E(%)
92,836	68,021	231	.003	73.0
92,836	73,366	1,382	.018	79.0
92,836	75,023	2,523	.033	81.0
92,836	79,500	3,975	.050	86.0
92,836	78,668	7,970	.100	85.0
92,836	80,718	13,005	.161	87.0
92,836	81,862	19,087	.233	88.2
92,836	82,160	21,270	.258	89.0
92,836	83,348	24,732	.296	90.0

If SCR $\leq$.050:E = 72.919 + 262.7258 SCR r^2 = .9691

If SCR >.050:E = 82.727 + 24.3955 SCR r^2 = .9869

Volumetric Productivity Estimates

Carbon uptake rates per liter or m^3 of lake water measure the fertility or algal productivity on a volumetric basis. Because of changes in light, and often temperature, within the photic zone; volumetric rates of photosynthesis change with depth often reaching peak values near the mid-euphotic zone depth. In addition, as the light compensation point often lies below the metalimnion, considerable algal photosynthesis can occur within the hypolimnion.

Calculations

Carbon (C-12) assimilation per cubic meter of lake water is estimated using: temperature, pH, alkalinity (carbon), and the C-14 DPM for each incubation bottle.

The C-12 assimilation rate is calculated using the expression:

$$\text{ug } C^{12}/l/hr = \frac{B \times C \times 1.06}{D}$$

B = Sample DPM divided by the total DPM added to the incubation bottle.

C = Available carbon (C-12) from alkalinity (as $CaCO_3$) x 1000 x CF (CF is the temperature and pH conversion factor obtained from Table 40).

D = Incubation time (hr).

1.06 = Isotope discrimination factor (C-12/C-14).

Algal C-12 incorporation or uptake rate is calculated using:

$$\text{Algal ug C-12/l/hr} = \bar{L} - \bar{\bar{D}}\bar{K}$$

$\bar{L}$ = Averaged C-12 uptake in light bottles

$\bar{\bar{D}}\bar{K}$ = Averaged C-12 uptake in dark bottle.

Example

The data printout (Table 41) for a single carbon-14 uptake experiment in Falls Lake, June 24, 1981 is used in the calculations. In addition, the following are necessary:

Temperature: 1 m = 12.4 , 5 m = 10.2, 10 m = 6.2
pH: 1 m = 5.9 , 5 m = 5.8, 10 m = 5.7
Alkalinity: 12 mg L^{-1} (as $CaCO_3$)
Carbon-14 added per bottle: 5.2 u Ci (11,569,925 DPM)
Incubation time: 1041 hr (T_o), 1441 hr (T_f)

For each incubation bottle, substitute the appropriate data into the carbon uptake expression as shown for the 1 m, light (L) bottle.

$$\text{ug C-12/l/hr} = \frac{6,203 \text{ DPM}}{11,569,925 \text{ DPM}} \times \frac{12,840 \text{ ug C-12/l} \times 1.06}{4.0 \text{ hr}} = 1.82$$

The carbon uptake rates for the remaining bottles comprising the 1-m depth are 1.45 (L), 0.35 (D), and 0.24 (D) ug C-12/l/hr. Finally, the algal carbon-12 uptake rate at the 1-m depth equals 1.34 ug C/l/hr. Similarly the uptake rates for the 5- and 10-m depths are 1.92 and 0.49 ug C/l/hr, respectively.

Table 40. Factors for the conversion of total alkalinity [mg L^{-1} ($CaCO_3$)] to milligrams of carbon per liter (Saunders et al. 1962).

	Temperature (C)					
pH	0	5	10	15	20	25
5.0	9.36	8.19	7.16	6.55	6.00	5.61
5.1	7.49	6.55	5.74	5.25	4.81	4.51
5.2	6.00	5.25	4.61	4.22	3.87	3.63
5.3	4.78	4.22	3.71	3.40	3.12	2.93
5.4	3.87	3.40	3.00	2.75	2.53	2.38
5.5	3.12	2.75	2.43	2.24	2.06	1.94
5.6	2.53	2.24	1.98	1.83	1.69	1.59
5.7	2.06	1.83	1.62	1.50	1.39	1.31
5.8	1.69	1.50	1.34	1.24	1.15	1.09
5.9	1.39	1.24	1.11	1.03	0.96	0.92
6.0	1.15	1.03	0.93	0.87	0.82	0.78
6.1	0.96	0.87	0.77	0.73	0.70	0.67
6.2	0.82	0.74	0.68	0.64	0.60	0.58
6.3	0.69	0.64	0.59	0.56	0.53	0.51
6.4	0.60	0.56	0.52	0.49	0.47	0.45
6.5	0.53	0.49	0.46	0.44	0.42	0.41
6.6	0.47	0.44	0.41	0.40	0.38	0.37
6.7	0.42	0.40	0.38	0.37	0.35	0.35
6.8	0.38	0.37	0.35	0.34	0.33	0.32
6.9	0.35	0.34	0.33	0.32	0.31	0.31
7.0	0.33	0.32	0.31	0.30	0.30	0.29
7.1	0.31	0.30	0.29	0.29	0.29	0.28
7.2	0.30	0.29	0.28	0.28	0.28	0.27
7.3	0.29	0.28	0.27	0.27	0.27	0.27
7.4	0.28	0.27	0.27	0.26	0.26	0.26
7.5	0.27	0.26	0.26	0.26	0.26	0.26
7.6	0.27	0.26	0.26	0.25	0.25	0.25
7.7	0.26	0.26	0.25	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.25	0.25	0.24	0.25
8.1	0.25	0.25	0.24	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24	0.24	0.24

Table 41. Liquid scintillation spectrometer data printout for a single carbon-14 uptake experiment in Falls Lake.

Depth (m)	Bottle type	RCPM	GCPM	SCR
1	L	5248.1	405.2	.077
1	L	4167.4	264.1	.063
1	D	949.3	21.6	.022
1	D	665.3	22.7	.034
5	L	5676.3	215.2	.038
5	L	4727.2	133.4	.028
5	D	1025.2	29.7	.028
5	D	881.1	17.7	.020
10	L	3626.7	71.5	.019
10	L	3662.0	133.3	.036
10	D	3002.5	161.0	.053
10	D	2677.1	52.7	.019

Day-Rate Estimates

Day rate estimates of photosynthesis are facilitated if the duration of experiments are timed proportional to day-length (see Appendix). Day-length is divided into five equal periods and by incubating in the second and third periods, 55%-60% of the total day rate is produced. Accordingly, the error introduced in estimating day-rate integrals of photosynthesis from exposure during the second and third periods will be of the order of $\pm$ 10% (Vollenweider, 1965). In order to avoid C-14 losses due to various processes (e.g., organic excretion) incubation should not exceed 4-6 hours.

Calculations

1) Divide sunrise to sunset into five equal periods by minutes, and incubate the samples for ~4-6 hours during the second and third periods (Figure 23).

2) Beginning with sunrise, add the number of minutes to T_o and to T_f, and locate each within their respective periods.

3) Determine T_o, and T_f as a percent of their respective periods, locate these percentages within each period (X-axis), and read percent cumulative productivity (Y-axis) for T_o and T_f.

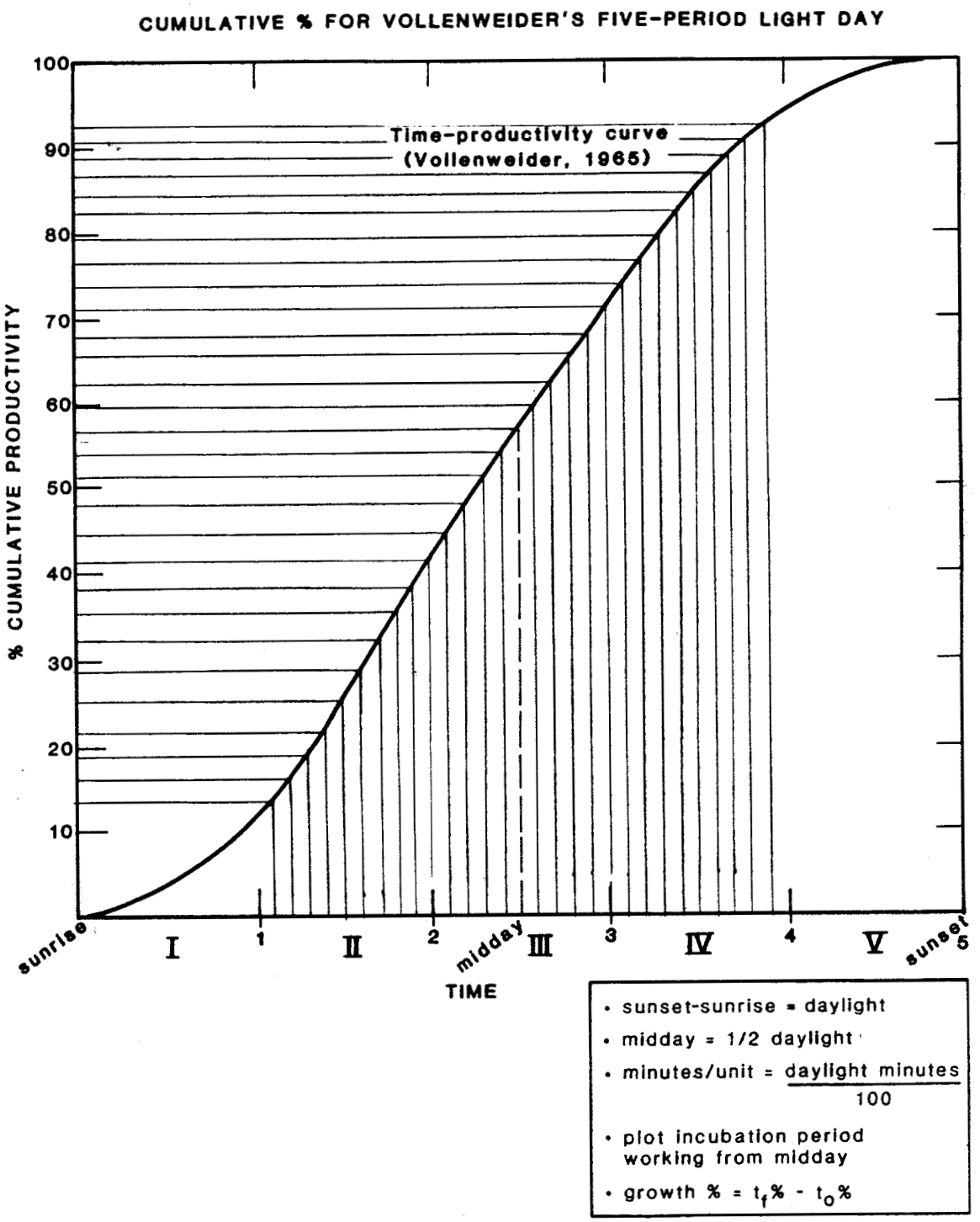

Figure 23. The relationship of cumulative primary productivity (expressed as a percent) to day-length divided into five equal periods (IV). Also shown are ten increments within each period (expressed as a percent) used to locate the initial and final carbon-14 incubation times, and the corresponding proportion (P) of cumulative productivity.

4) Subtract the percent cumulative productivity found for T_o from that found for T_f, which equals the percent (P) of the total daily carbon-12 assimilated during the incubation period.

5) Determine the amount of carbon-12 (mg C-12/m^3) assimilated during the incubation period by multiplying the volumetic carbon-12 assimilation rates (p. 137) by the duration of incubation (hours).

6) Divide mg C-12/m^3 by the proportion (P/100) of the daily rate to obtain the day-rate estimate expressed as mg C-12/m/d.

NOTE: Vollenweider (1965) determined that the specific shape of the photosynthesis depth curve is of much less importance with regard to day-rate estimates than had generally been thought. Nonetheless, C-14 in-situ exposure should take place at ~40% of the incident solar radiation when using single depth determinations.

Example

During the Falls Lake experiment the total day-length on June 24 was 1097 minutes (sunrise 0352 hr, sunset 2209 hr), and the five resultant 219 minute periods are:

Period 1 = 0- 219 minutes
Period 2 = 220- 438 minutes
Period 3 = 439- 658 minutes
Period 4 = 659- 878 minutes
Period 5 = 879-1,097 minutes

The incubation began (T_o) at 1041 hours or at 408 minutes after sunrise. The incubation ended at 1441 hrs or at 649 minutes after sunrise. T_o (408) falls at 86% (408-220/219) of period 2, and T_f (649) falls at 96% (649-439/219) of period 3.

T_o corresponds to a percent daily rate equal to 38% (Figure 23) and T_f corresponds to 68% of the daily rate. Thus, the percent of the day-rate productivity measured in this experiment was 30% (68%-38%). Finally to estimate the day-rate productivity divide the total amount of algal carbon uptake by 0.3 (30%/100).

The day-rate estimate equals:

$$\frac{1.29 \text{ mg C/m}^3\text{/hr} \times 4 \text{ hr}}{0.3} = 17.2 \text{ mg C/m}^3\text{/day} = 17.2 \text{ ug C/l/day}$$

Similarly the day-rate estimates for the 5- and 10-m depths are 25.1 and 6.5 mg C/m^3/day respectively.

Areal Estimates

Areal primary productivity is defined as the rate of autotrophic biomass accural under a unit area lake surface, and is expressed as mg $C/m^2/day$.

Procedure

1) Plot day-rate estimates (mg $C\text{-}12/m^3/day$) on the X-axis versus depth on the Y-axis, and draw a smooth curve through the points (Figure 24).

2) Calibrate the planimeter by measuring a known area (mg $C/m^3/day$ x m) to obtain a conversion factor of planimeter readings to known area (see Instrument Calibrations, p. 192).

3) Measure the area within the curve using a calibrated planimeter, and convert planimeter readings to areal productivity (mg $C\text{-}12/m^2/day$) by multiplying by the conversion factor (Step 2).

Example

The day-rate estimates for Falls Lake (June 24, 1981) equalled 17.2 (1 m), 25.1 (5 m), and 6.5 (10 m) mg $C/m^3/day$ (Figure 24). The area within the curve is represented by 317 planimeter units, and when multiplied by the conversion factor of 0.6152 resulted in an areal productivity of 195 mg $C/m^2/day$.

If a planimeter is unavailable, two alternative methods are:

1) Sum the mg $C/m^3/day$ for each 1-m layer (where the area within each 1-m layer equals mg $C/m^3/day$ x 1 meter or mg $C/m^2/day$) using the mid-strata value for each layer (Figure 24).

2) Average the day rate estimates at the various depths, and multiply by the depth of the euphotic zone.

Finally, areal productivity estimates using the three methods were compared for Falls Lake (June 24, 1981) (Figure 24), and for three lakes with varying photic zones (Figure 25).

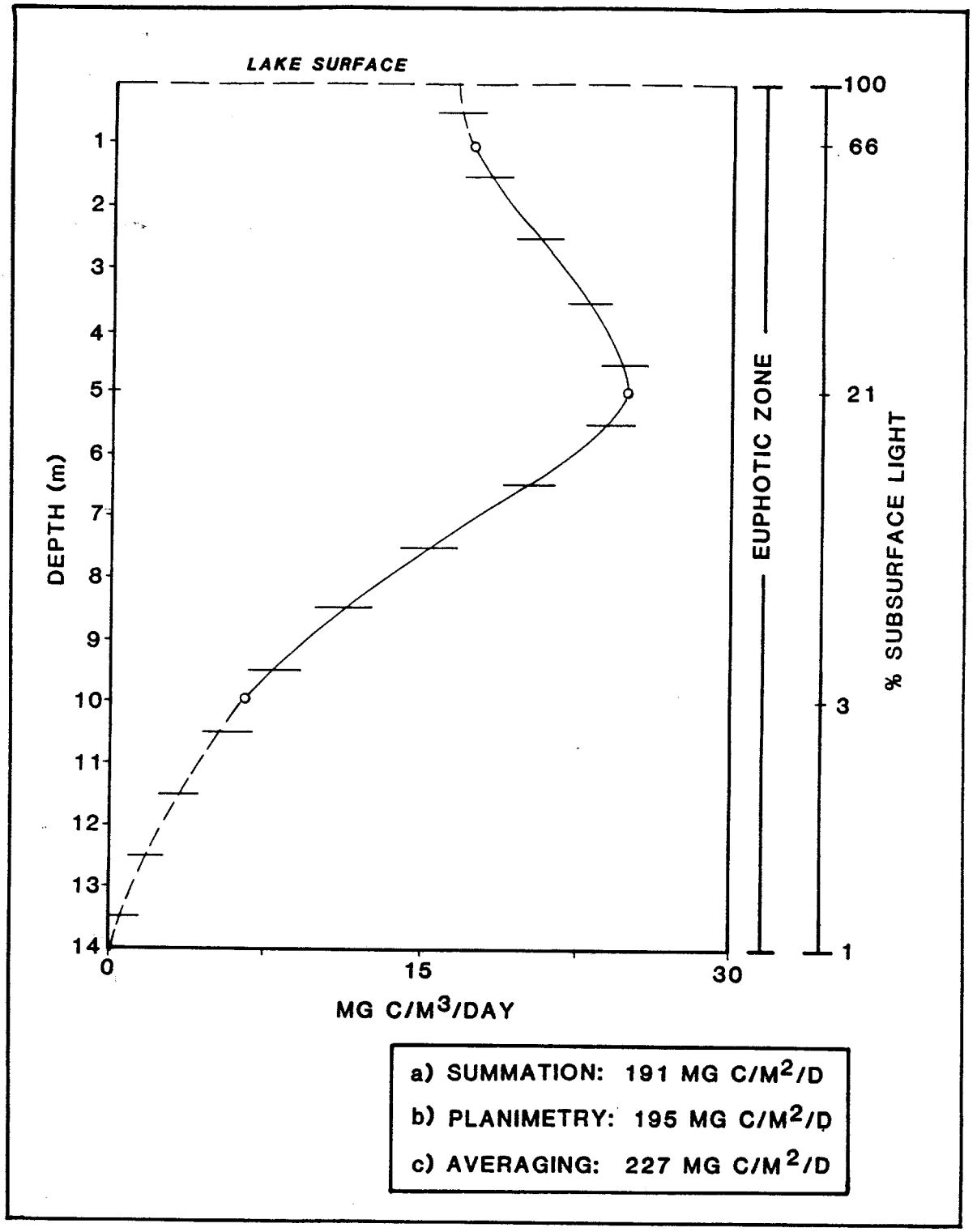

Figure 24. Vertical distributions of day-rate primary productivity estimates within the euphotic zone used in three procedures to obtain areal productivity (mg C/m^2/day) in Falls Lake, 24 June 1981.

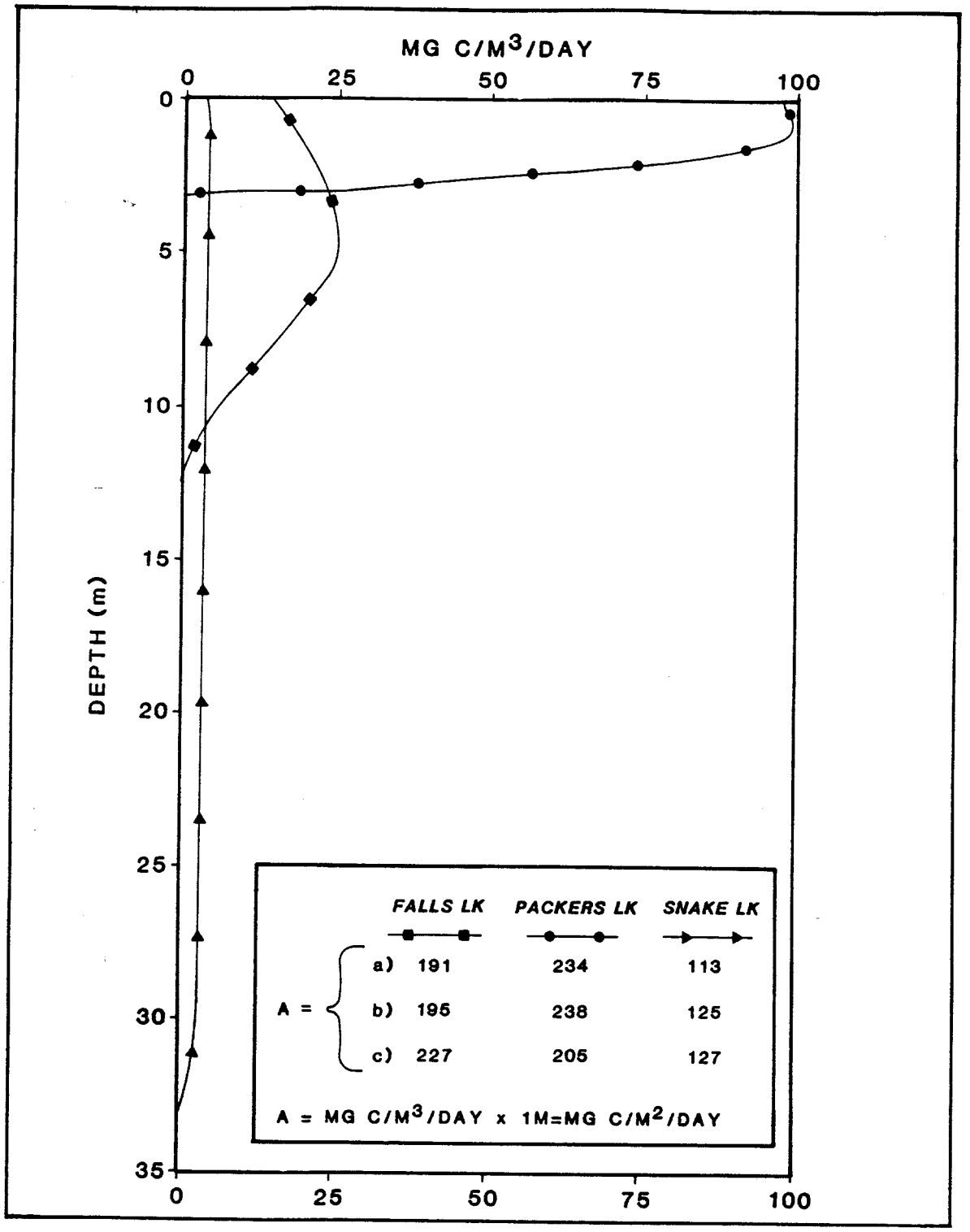

Figure 25. Vertical distributions of day-rate primary productivity estimates (mg $C/m^2/day$) in three lakes, Falls, Packers, and Snake, with varying photic zones; and a comparison of areal productivity estimates (A) as determined by summation (a), planimetry (b), and averaging (c).

PRIMARY PRODUCTION

Phytoplankton standing crop can be represented by the concentration of algal pigments. The composition of the algal pigments varies depending upon physiological status and the relative classes making up the phytoplankton community. However, only chlorophyll a (chl a) is considered here since concentrations of chl b and chl c are negligible by comparison.

Chlorophyll samples rapidly degrade to phaeophytin a (phaeo a) upon changes in light, temperature, and pH. As the inactive phaeo a pigment absorbs and fluoresces light at the same wavelengths as chl a, large amounts of phaeo a bias estimates of chl a. Thus, both the spectrophotometric and fluorometric analyses require an acidification step to correct for phaeophytin. By taking spectrophotometric and/or fluorometric measurements before and after acidification, estimates of both chl a and phaeo a concentrations can be made.

The procedures for determining chl a and phaeo a are modified from Strickland and Parsons (1972). Chl a is extracted from algal cells after grinding the filter in 90% acetone. The absorbance of the extraction solution is then measured before and after acidification with dilute HCl (Reimann 1978).

Chlorophyll a and Phaeophytin a (spectrophotometric)

Limit of Detection

0.04 ug L^{-1} (lake concentration).

Apparatus

Spectrophotometer (750 mm, 665 nm, 663 nm, 645 nm, 630 nm), tissue grinder, teflon serrated pestle, grinding vessel, centrifuge, 15-ml glass centrifuge tubes, and 10-mm cuvettes.

Reagents

1) 90% neutralized acetone - Dilute 900 ml of reagent-grade acetone to 1 liter with DI water, add 1 ml of 1 N $NaHCO_3$, and filter through a 'fast' pleated filter.

2) 2 N HCl - Dilute 42 ml of concentrated hydrochloric acid to 250 ml with DI water.

3) 1 N Sodium bicarbonate - Dissolve and dilute 8.4 g $NaHCO_3$ into 100 ml DI water.

Procedure

1) Thaw filters, place into a grinding vessel, and add ~2 ml of 90% acetone.

2) Grind while moving the vessel up and down against the serrated pestle until the contents form a slurry.

3) Rinse the pestle with ~4 ml of 90% acetone, transfer the slurry into a 15-ml centrifuge tube, rinse the vessel with ~4 ml 90% acetone, and add the contents to the centrifuge tube.

4) Cover with aluminum foil, and refrigerate the samples for 2-3 hours to complete chl *a* extraction.

5) Centrifuge for 40 minutes at 2500 rpm, and decant the supernatant into another 15-ml centrifuge-tube, and dilute to 12 ml with 90% acetone.

6) Invert, split into two equal parts, and to one tube add 0.05 ml of 2 N HCl. Invert the acidified tube to mix.

7) Measure the absorbances 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 663 nm.

NOTE: While analyzing keep the samples in the dark or under subdued light as much as possible. Wash all glassware with 90% acetone and avoid using 10% HCl.

Calculations

The trichromatic and monochromatic methods can both be used to determine chlorophyll concentration. Careful attention must be given to the interpretation of the results. The trichromatic method estimates total pigment (chl *a* plus phaeo *a*); whereas the monochromatic method estimates chl *a* and phaeo *a* separately. The trichromatic equation (SCOR/UNESCO) uses absorbances before acidification, and is modified here to yield in-lake pigment concentration.

$$\text{chl } a + \text{phaeo } a \text{ (ug L}^{-1}) = \frac{[11.64(663_o)-2.16(645_o)+.10(630_o)]Vs}{L \times Vf}$$

(663_o) = absorbance 663 nm - absorbance 750 nm
(645_o) = absorbance 645 nm - absorbance 750 nm
(630_o) = absorbance 630 nm - absorbance 750 nm
Vs = total volume (ml) of sample extract (Step 5).
L = pathlength (cm) of cuvette.
Vf = volume (l) of lake water filtered.

The monochromatic method (Strickland and Parsons 1972) uses separate equations for chl *a* and phaeo *a*. These equations are both modified to yield in-lake pigment concentrations.

$$\text{chl a } (\text{ug } L^{-1}) = \frac{26.7\ (665_o - 665_a) \times Vs}{L \times Vf}$$

$$\text{phaeo } \underline{a} \text{ } (\text{ug } L^{-1}) = \frac{26.7\ [1.7\ (665_a) - 665_o] \times Vs}{L \times Vf}$$

(665_o) = absorbance 665 nm - absorbance 750 nm (before acidification).
(665_a) = absorbance 665 nm - absorbance 750 nm (after acidification).
Vs = total volume (ml) of sample extract (Step 5).
L = pathlength (cm) of cuvette.
Vf = volume (l) of lake water filtered.

As periphyton samples (see p. 10) often contain very high chl *a* concentrations, we recommend using the spectrophotometric (monochromatic) method in order to avoid large dilutions of the sample extract. Periphyton chl *a* and phaeo *a* concentrations are reported as mg/m^2 by removing the volume filtered (Vf) from the monochromatic equations and substituting the area (m^2) of the plexi-glass plates.

Chlorophyll a and Phaeophytin a (fluorometric)

Algal pigments extracted in acetone fluoresce when subjected to a specific wavelength of light. Instrument readings both before and after acidification are required to determine chl *a* and phaeo *a* levels. The fluorometric method is preferred because of its increased sensitivity. Pigment concentrations are based on sample fluorescense, relative to known chl *a* standards.

Limit of Detection

0.04 ug L^{-1} chl *a* (lake concentration), 0.05 ug L^{-1} phaeo *a*.

Apparatus

Turner model 111 or 112 filter fluorometer equipped with a F4T5-B source lamp, Corning CS 5-50 excitation filter (420 nm) and a Corning CS2-64 emission filter, tissue grinder, serrated pestle, centrifuge, 0.05-ml autopipet, 15-ml centrifuge tubes, 12- x 75-mm borosilicate-glass culture tubes (cuvettes).

Reagents

Prepare the reagents as described in the spectrophotometric method (p. 146).

Procedure

1) Follow steps 1-5 of the spectrophotometric procedure (p. 147).

2) Calibrate the fluorometer (see Instrument Calibrations, p. 192).

3) Pour ~4 ml of the extract into a glass culture tube, and record fluorescense units (Rb) and the appropriate sensitivity setting (S). If the reading is greater than 100 on the S_1 window, dilute the sample with 90% acetone, and calculate a dilution factor (DF). If the reading is less than 10, increase the sensitivity setting.

4) Acidify the extract in the culture tube with two drops of 2 N HCl using a 0.05 ml autopipet, and mix. Wait 30 seconds, record the fluorescence (Ra) and the appropriate sensitivity setting.

Calculations

In-lake concentrations of chl *a* and phaeo *a* are calculated using:

$$\text{Chl } a \text{ (ug L}^{-1}) = \frac{Sx(Rb-Ra)(.012)\ (DF)}{VF}$$

$$\text{Phaeo } a \text{ (ug L}^{-1}) = \frac{Sx[(rs \times Ra)-Rb](.012)\ (DF)}{VF}$$

Sx = calibration factor.
rs = acid ratio.
Rb = fluorescence units before acidification.
Ra = fluorescence units after acidification.
DF = dilution factor.
VF = volume (l) of lake water filtered.

In summary, there are three methods that can be used to analyze for total pigment, chlorophyll *a* and phaeophytin *a*. These are

the (1) trichromatic, (2) monochromatic, and (3) fluorometric methods (Table 42). Low chlorophyll *a* levels should be determined using the fluorometric method, while higher (>10 ug L^{-1}) in-lake concentrations, and chl *a* in macrophytes and periphyton should be analyzed using spectrophotometric methods.

Table 42. Comparison of total pigment, chlorophyll *a* (chl *a*), and phaeophytin *a* (phaeo *a*) levels as determined by the trichromatic, monochromatic and fluorometric methods.

	Trichromatic	Monochromatic		Fluorometric	
Sample	Total pigment	Chl a	Phaeo a	Chl a	Phaeo a
Grant Lake	0.21	0.16	0.06	0.11	0.15
Packers Lake	1.35	1.28	0.06	1.24	0.67
O'Malley Lake	4.35	3.52	1.60	2.75	2.23
Bear Lake	18.68	20.8	<0.04	16.52	1.13
EPA spectrophotometric reference	7200	5794	2561	6024	4453
Sigma reference	2736	2830	<0.04	2806	<0.04
Macrophyte extract	1.14	1.12	0.4	0.78	1.01

NOTE: The results are expressed as ug L^{-1} with the exception of the macrophyte extract which is expressed as mg per g dry weight of plant material.

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SECONDARY PRODUCTION

The limited littoral area typical of sockeye salmon nursery lakes emphasizes the importance of limnetic zooplankton production for juvenile survival and growth. As the zooplankton community serves as the primary forage base for sockeye juveniles, the characteristics of zooplankton populations are important indicators to gauge either present or potential rearing (forage) conditions for limnetic feeding salmon fry; and to evaluate results of fry stocking and nutrient enrichment programs. Important zooplankter characteristics include species or community composition, body-size, biomass, and seasonal timing and fluctuations in density. The amount of zooplankton that is actually available as food for rearing salmon fry is often a sub-fraction of the total because fry are very selective feeders. By electing to feed on certain species and body-sizes, fry exert a predation pressure that can structure zooplankton communities. In general, zooplankter communities with few or small cladocerans are consistent with a heavy predator pressure. In contrast, communities with high densities of large cladocerans may have little or no predatory pressure from fish.

Such generalizations need to be applied with caution, as recent studies (Gliwicz 1986, Koenings et al. 1986) have shown that environmental conditions can also reduce or eliminate cladocerans from the zooplankton community. Appropriate fish enhancement strategies can be formulated from a knowledge of how fish predation and other components of the environment structure zooplankton communities.

Zooplankter Identification

The zooplankton community consists of three major groups, cladocerans, copepods, and rotifers. Each has evolved different life history strategies in response to predator pressures, food availability, and environmental stimuli. Briefly, rotifers are small, non-prey items for salmonids, and have a rapid reproductive rate. Cladocerans also have a rapid reproductive rate; but are larger, non-evasive and are preferred prey items for salmonids. The copepods are of similar size to the cladocerans; but have a slower reproductive rate, are extremely evasive, and are only occasional forage for salmonids. That is, cladocerans survive by simply out-producing predators while copepods simply out-maneuver them.

Zooplankters are identified to species using either a stereo dissecting or compound microscope, and various taxanomic keys. Cladocerans can be easily identified using only a dissecting microscope. However, copepods must be speciated using adult

specimens with identifying parts dissected out, mounted on glass slides, and examined under a compound microscope.

Apparatus

Bausch and Lomb stereo dissecting microscope, 1-ml Hensen-Stemple pipet, 1-ml Sedgewick-Rafter counting cell, and taxanomic keys for freshwater invertebrates.

Procedure

1) Place a section of 130-u mesh plankton net into a funnel, and empty the contents of the sample bottle onto the net. Rinse the sample bottle several times with tap water and pour onto the net.

2) Invert the net over a 250-ml beaker, and using a wash bottle rinse into the beaker. The zooplankters are now formalin free and can be diluted to a known volume with tap water for density estimates (see below).

3) Withdraw a 1-ml aliquot of sample using a Hensen-Stemple pipet, add to a Sedgewick-Rafter counting cell, dissect for identification if necessary, and place under the appropriate microscope.

4) Identify Daphnia using Brooks (1957), Bosmina and other cladocerans using Brooks (1959), and copepods using Wilson (1959), Yeatman (1959), and Harding and Smith (1974).

   NOTE: The common cladocerans found in Alaskan lakes are Bosmina, Daphnia, Holopedium, Chydorus, and Polyphemus. The copepods include Cyclops, Diaptomus, and Epishura. Rotifers include Kellicottia, Asplanchia, Karetella, Conochiloides, and Filinia.

5) Replace the tap water in the beaker with 10% neutralized formalin if the sample is to be reused or stored.

Volumetric and Areal Density Estimates

Density estimates measure standing crop at any moment in time, and differ from rates of biomass accrual or production measured overtime. As such, standing crop estimates represent a balance between removal by predators and accrual by reproduction. Zooplankters peak at different times (in response to environmental stimuli), occupy different depths in the water column, and vary in susceptibility to vertebrate and invertebrate predation pressures. Thus, synchrony of peak

densities of zooplankters peferred by limnetic feeding fry is critical for optimizing freshwater growth and survival.

In-lake density estimates are derived from counting zooplankters within 1-ml subsamples withdrawn from a known volume of preserved sample; and from either the volume (m^3) of lake water filtered or diameter (m^2) of the zooplankton net. Areal density (per m^2) estimates the number of zooplankters beneath one square meter of lake surface regardless of vertical distribution in the water column. Volumetric density (per m^3) estimates imply an equal dispersion of the zooplankters throughout the water column.

Apparatus

Bausch and Lomb stereo dissecting microscope, 1-ml Hensen-Stemple pipet, 1-ml Sedgwick-Rafter counting cell.

Procedure

1) See Zooplankter Identification procedure Steps 1 and 2 (p. 152).

2) Dilute the contents of the beaker with tap water so that a 1-ml aliquot will contain ~100-150 organisms.

   NOTE: If the 250-ml dilution is still too concentrated, the sample must be split or divided into equal parts.

3) Connect a number (~7) of triangular containers together and place on a record turntable. Pour the sample into a closed funnel, and rinse the beaker with 50 ml of tap water.

4) Rotate the turntable, open the funnel, and allow the sample to distribute into the containers.

5) Remove any three containers, pour into three beakers, and dilute each to a known volume.

6) Mix, withdraw a 1-ml subsample with a Hensen-Stemple pipet, and expel onto the Sedgewick-Rafter cell. Apply a cover glass, count all the plankters in the cell, and return the subsample to the beaker.

7) Count a total of three 1-ml aliquots, and if the sample is to be saved, reconcentrate and preserve.

Calculations

1) Average the number of each species per ml and multiply by the total volume (ml) of subsample. If the original

250-ml sample was split, first multiply by the number of containers (~7) and then by 250 ml.

2) Divide by the number of tows to obtain the number of plankters per tow.

3) Divide the plankton per tow either by the area (m^2) of the net opening or by the volume (m^3) towed:

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

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

General Notes

a) The counts from the three 1.0-ml subsamples (aliquots) should be similar. If one count differs, recount an additional subsample, and substitute for the biased subsample.

b) If the 1-ml subsample contains less than 100-150 zooplankters, additional counts from a concentrated sample may be necessary to achieve consistent counts.

c) Count copepod egg sacs, and report as a separate total.

d) Etched lines on the Sedgewick-Rafter cell facilitate systematic counting.

Body-Size (length) Measurements

Macro-zooplankters ranging in body-size (length) from 0.2 to >2.0 mm are represented by cladocerans and copepods. Juvenile fish feeding on large zooplankters grow faster and more efficiently; however, continual pressure from size selective predation restructures the zooplankton community. That is, the remaining zooplankters are small-sized and are species that are more evasive. Both responses confer a resilience to further predation. Moreover, as zooplanker body-sizes are lowered, fecundity drops, and densities decline. Thus, zooplankton communities in lakes with planktivorous fish differ substantially from communities in similar lakes without planktivores; and the standing crop remaining after predation reflects both the degree of predator pressure and the feeding nature of the predator.

Body-size measurements are made with an ocular micrometer using a stage micrometer and the power-magnification adjustment for calibrating the micrometer.

Apparatus

Bausch and Lomb stereo dissecting microscope, ocular micrometer, stage micrometer, 1-ml Hensen-Stemple pipet, and a 1-ml Sedgwick-Rafter counting cell.

Procedure

1) Calibrate the ocular micrometer (see Instrument Calibrations, p. 192) for the appropriate (0-2 mm or 1-10 mm) scale.

2) In each of three 1-ml subsamples, measure the distance from the top of the head to the end of the carapace (Figure 26) of the first five individuals of each species encountered.

3) Record lengths to the nearest 0.01 mm when using the 0-2 mm scale and to the nearest 0.1 mm when using the 1-10 mm scale.

4) Determine the required number of organisms to measure with a precision of ± 10% at the 95% confidence level using the following:

   a) Calculate the mean length (L) and standard deviation (SD) of the first 15 zooplankters of a species, and determine n by substituting into the formula:

$$n = [(1.96 \times SD)/(.10 \times L)]^2$$

   b) Determine the number (N) of zooplankters to be measured by using n-1 and the t-statistic at a confidence level of 95% (Table 43), and substituting t, SD, and L into the formula:

$$N = [(t \times SD)/(.10 \times L)]^2$$

$$N = \text{number to be measured}$$

Calculation

Determine a weighted mean length, from the numbers of zooplankters within each size class (0.5 mm intervals), per species for each set (2) of samples.

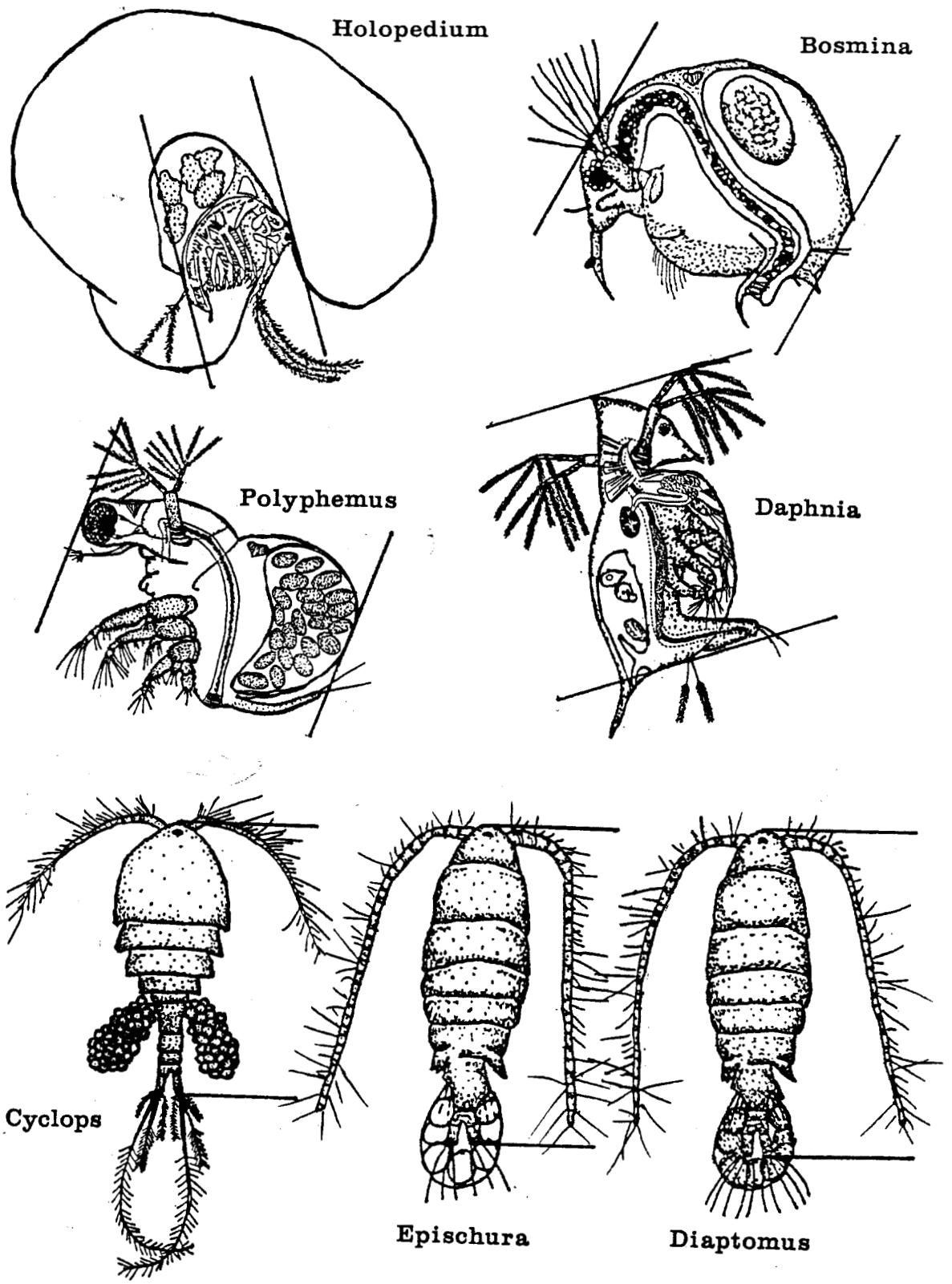

Figure 26. Location of anterior and posterior measuring points on the carapace of cladocerans, and cyclopoid and calanoid copepods used to determine body-size (length).

Table 43. Student's t-statistic and sample sizes (n) used to determine the number (N) of zooplankters 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	2.07
8	2.31	18	2.10	28	2.05
9	2.26	19	2.09	29	2.05
10	2.23	20	2.09	$\geq$30	1.96

Individual Body-Weight (biomass) Estimates

As zooplankton biomass is dependent on both the body-size (weight) and density (numbers), seasonal changes in density may not be consistent with changes in biomass. Since the zooplankton community serves as the primary forage base for rearing sockeye juveniles, the carrying capacity of lakes is dependent on the biomass of the zooplankton. More precisely, rearing capacity depends on the biomass of the specific zooplankters that are used by rearing juveniles. As this may be only a fraction of the total, the biomass of only the preferred prey species can serve as an index to carrying capacity. Individual zooplankters are routinely identifed, enumerated, and sized; but not weighed because of time constraints. However, from an empirical correlation between individual dry weights and body-sizes (wet); routine size measurements can be converted to weights and then to biomass.

Individual zooplankters of each species are dried, and then weighed on a Mettler UM3 micro-balance to the nearest 0.0001 mg. The resulting regression equations, between each zooplankter length and dry weight are used to calculate zooplankter biomass from individual lakes.

Apparatus

Mettler UM3 micro-balance, Basch and Lomb dissecting microscope, 1" x 3" glass microscope slides, and needle probes.

Procedure

1) Place a representative size range of individual zooplankters of known lengths onto a labeled 1" x 3" microscope slide, and carefully agitate while drying (~3-4 minutes) with a needle probe (~10 zooplankters can be placed on a slide).

2) Place an individual zooplankter on the balance pan using the needle probe, and weigh to the nearest 0.0001 mg.

3) Record dry weights along with corresponding wet lengths for each zooplankter.

Calculations

1) Regress individual dry weights (mg) (Y-axis) against length (mm) (X-axis), formulate either a linear or a power curve, and calculate the coefficient of determination (r^2) for each species (Figure 27).

2) To calculate biomass, substitute the weighted mean length (mm) of each species into the appropriate regression formula (Table 44), calculate the mean weight (mg) of each species, and multiply by areal densities (Table 45).

Table 44. Regression equations showing the relationship between individual body-size (mm) and dry weight (mg) for seven zooplankter species.

Species	Regression equation	Coefficient of determination (r^2)
Bosmina longirostris	$mg=-0.0033+0.0118(mm)$	0.80
Daphnia longiremis	$mg=0.0043(mm)^{2.26}$	0.84
Holopedium gibberum	$mg=0.0114(mm)^{2.44}$	0.81
Diaptomus pribilofensis	$mg=0.0042(mm)^{2.77}$	0.91
Epischura nevadensis	$mg=0.0039(mm)^{3.05}$	0.93
Cyclops columbianus	$mg=0.0036(mm)^{1.99}$	0.84
Cyclops vernalis	$mg=0.0048(mm)^{3.01}$	0.82

3) Sum the biomass of each species to yield the total dry weight biomass (mg/m^2) of the zooplankton.

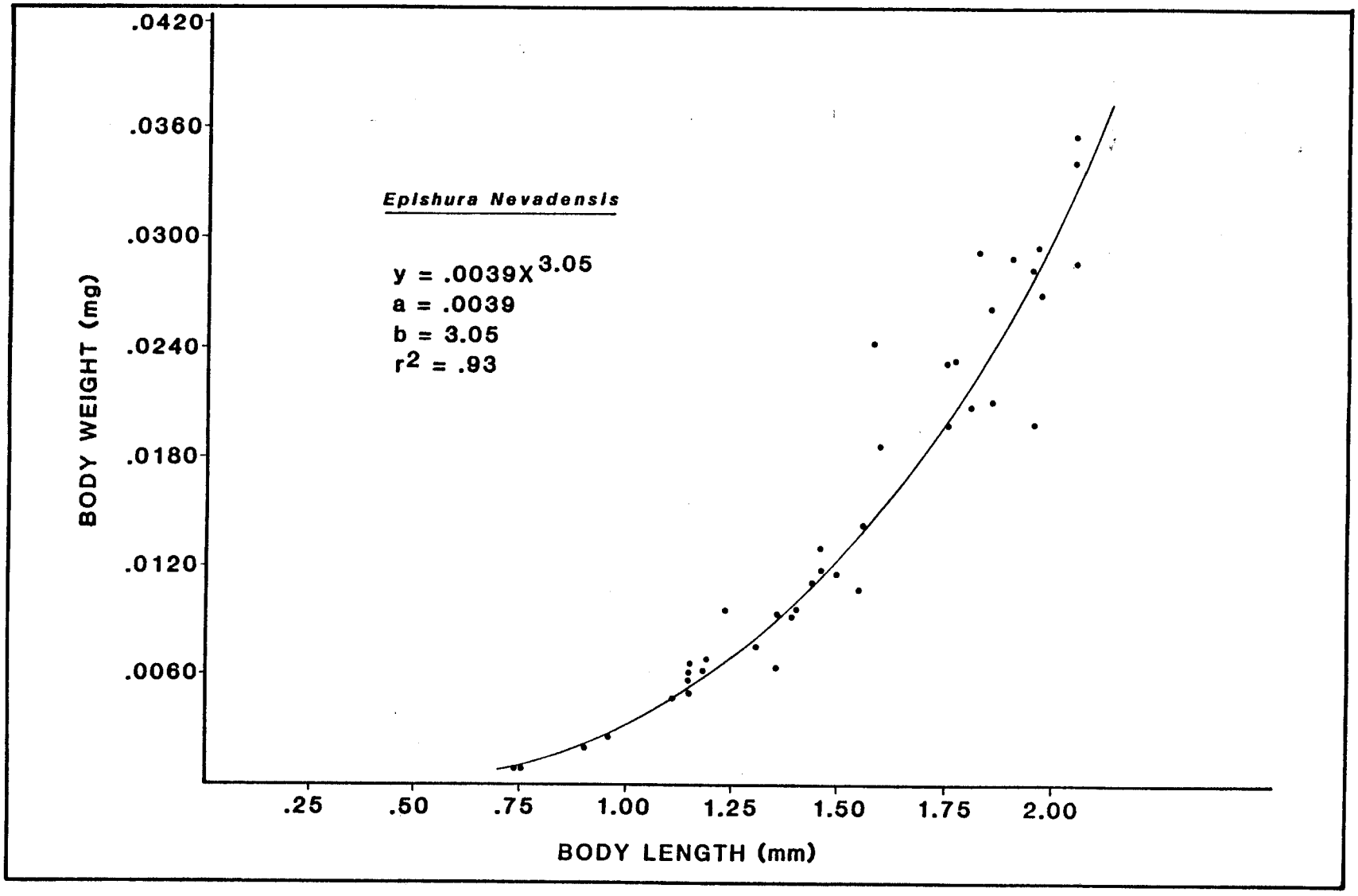

Figure 27. The relationship between dry weight (mg) and wet length (mm) for Epishura nevadensis derived using measured zooplankters from both Hidden and Packers Lakes.

Table 45. In-lake zooplankter biomass (mg/m^2) calculated using density estimates, and the relationship between zooplankter dry weight and mean length at Packers and Hidden Lakes.

Lake	Date	Mean wet length (mm)	Mean dry weight (mg)	Density (number/ m^2)	Dry weight biomass (mg/m^2)
		Daphnia longiremis			
Packers	06/06/84	0.68	.0018	18,047	32.5
Packers	06/27/84	0.96	.0040	77,495	310.0
Packers	06/30/84	0.89	.0030	81,476	244.4
		Epishura nevadensis			
Hidden	06/25/8	1.42	.0114	50,375	574.3
Hidden	07/16/8	0.84	.0018	5,921	10.7
Hidden	09/01/8	1.13	.0060	2,400	14.4
		Diaptomus pribilofensis			
Packers	06/06/84	0.71	.0016	131,370	210.2
Packers	06/27/84	1.00	.0042	180,467	758.0
Packers	08/30/84	1.15	.0063	87,315	550.0
		Bosmina longirostris			
Packers	06/06/84	0.36	.0010	16,189	16.2
Packers	09/19/84	0.44	.0018	8,623	15.5
Packers	10/11/84	0.50	.0021	2,389	5.0

Total Wet/Dry Weight (biomass) Estimates

Material retained by a 130 u mesh zooplankton net includes macro- and micro-zooplankters as well as detritus and filamentous algae which bias weights determined directly from the netted material. However, in oligotrophic systems zooplankton are considered to constitute a majority of the total weight so actual bias may be minimal.

The biomass of the total zooplankton community is estimated from the dry weight of organisms retained on a preweighed filter, and is expressed on the basis of net area or towed volume.

Apparatus

Vacuum pump (≤15 psi), analytical balance with a resolution of 0.0001 g, Millipore RAWP 04700 filters, aluminum weighing pans or small glass petri-dishes, drying oven (80 C), filter forceps, and a desiccator.

Procedure

1) Place a filter on the balance pan using blunt filter forceps, record the weight (W_d), and place the filters in numbered weighing pans.

2) Using the filtering apparatus (Figure 3), draw ~100 ml of tap water through the filter at a vacuum of ≤15 psi, weigh (W_w), and return to the numbered weighing pan.

   NOTE: Using RAWP filters, we have found a <10% difference in weight between filters that are hydrated, dried, and desiccated; and untreated filters.

3) Remove the formalin by straining a sample through a section of 153-u mesh netting, and rinsing with tap water.

4) Remove the zooplankton from the net by inverting over a beaker, and rinsing with ~50 ml of tap water.

5) Filter the contents of the beaker through a pre-weighed (W_d and W_w) filter, and rinse the tower with a small volume of water.

6) Remove the filter and weigh, record the weight (W_{wf}), and return to the numbered weighing pan.

7) Place the weighing pan into the drying oven at 80 C for ~3 hours, and cool in a desiccator for ~2 hours.

8) Weigh the desiccated filter and record the weight (W_{df}).

   NOTE: Weigh immediately after desiccation as the filter rapidly adsorbs moisture from the air.

Calculations

1) Determine wet and dry weights using the following:

$$\text{Wet weight (g)} = W_{wf} - W_w$$

$$\text{Dry weight (g)} = W_{df} - W_d$$

2) Divide the wet and dry weights by the area of the net opening (m^2) and by the volume (m^3) of lake water filtered to obtain biomass estimates.

3) Determine the percent moisture content (Table 46) using the following:

$$\text{Moisture content (\%)} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

Table 46. Comparison of wet and dry weights, and the moisture content of the zooplankton communities in four Alaskan lakes during the spring, summer, and fall periods.

Lake	Date	Wet weight (mg)	Dry weight (mg)	Moisture content (%)
Karluk	05/10/86	82	8	90
	06/28/86	621	54	91
	11/21/86	305	29	90
Redoubt	05/10/83	46	2	96
	07/16/83	164	23	86
	11/20/83	46	3	93
Packers	03/06/81	54	1	98
	07/09/81	430	48	89
	10/13/81	203	21	90
Frazer	05/14/86	62	3	95
	08/28/85	258	19	93
	10/02/85	213	11	95

Reference Slides

Reference slides are used to confirm the identification of zooplankters, particularly the copepods. Reference sllides are sent to the Smithsonian Institution, Washington D.C. for zooplankter identification, and have been added to the Smithsonian's permanent reference collection.

Apparatus

4-dram glass vials, microscope slides, cover slips, needle probe, disposable pasteur pipets, and a hot plate.

Reagents

1) Rose Bengel - Dissolve ~0.2 g of Rose Bengel stain (C.I. 45440) into 100 ml of DI water.

2) Mounting medium - Permount, Fisher Scientific Company.

3) 70% Reagent alcohol - Dilute 70-ml of reagent alcohol-anhydrous to 100-ml with DI water.

4) Glycerin

5) Glycerin jelly

Procedure

1) Add ~2 drops of Rose Bengel to cover zooplankton in a glass vial, and stain overnight.

2) Pour off the excess stain, add tap water, decant, and repeat until clear.

3) Place the specimens in 70% reagent alcohol, add liquid glycerin to make a 20% solution, and leave the vial uncapped until the alcohol and water evaporate (~ 2 days).

4) Place a specimen on a microscope slide, add one drop of liquid glycerin, add one or two drops of warmed glycerin jelly, and quickly realign the specimen using a warm needle probe.

5) Cover with a warmed cover slip, allow to congeal for 30 minutes, and cement the outer edge of the cover slip with Permount.

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JUVENILE SALMON EVALUATION

Total Body Burden of Oxytetracycline

The antibiotic oxytetracyline (OTC) is added to hatchery fish food and used as a prophylactic medicine prior to release. Once digested, OTC is absorbed and deposited in the skin, cartilage, and skeleton of juvenile salmon. OTC held in bone as a calcium-complex is retained for the life of the fish. Koenings et al. (1986) have shown the feasibility of using OTC as an internal mark for hatchery-reared sockeye salmon.

OTC extracted from biological material using trichloroacetic acid (TCA) forms a fluorescent complex with calcium that is measured by fluorometry.

Standard Range

0.47 - 9.4 ug OTC.

Lower Limit of Detection

Empirical: 0.031 ug ml^{-1}.
Predicted: 0.012 ug ml^{-1}.

Apparatus

Shaker table, centrifuge, spectrofluorometer (excitation 390 nm; emission 520 nm) 50-ml centrifuge tubes with teflon lined caps, blender with micro-cell, and disposable 15-75 mm borosilicate culture tubes (cuvettes).

Reagents

1) 5% TCA - Dissolve 50 g of trichloroacetic acid (TCA) into 900 ml of DI water, and dilute to 1 liter.

2) Ethylacetoacetate - Ultrapure.

3) Ethylacetate solution - Dilute 13 ml of ethylacetoacetate to 1 liter with ethyl-acetate.

4) 0.5 M $CaCl_2$ - Dissolve 36.7 g of $CaCl_2$ into 450 ml of DI water, and dilute to 500 ml.

5) 7 N NH_4OH - Dilute 437.5 ml of concentrated reagent-grade NH_4OH to 1 liter with DI water.

Procedure

1) Homogenize individual fish in 15 ml of 5% TCA using a blender, pour into a 50-ml centrifuge tube, and rinse the slurry using 5 ml of TCA into the tube.

   NOTE: Soak centrifuge tubes overnight in 10% HCl, thoroughly wash with phosphate-free detergent, and rinse with 10% HCl.

2) Place the tubes on a shaker table, and agitate for 1 hour in the dark.

3) Centrifuge at 2000 rpm for ~30 minutes, pipet 15 ml of the TCA extract into a clean 50-ml centrifuge tube, and add 15 ml of ethylacetate solution.

4) Agitate on a shaker table ~10 minutes, and centrifuge at 2000 rpm for 5 minutes.

5) Withdraw the top layer using an autopipet, place into another 50-ml centrifuge tube and add 0.5 ml of 0.5 M $CaCl_2$ followed by 15 ml of 7 N NH_4OH.

6) Agitate for ~10 minutes and centrifuge at 2000 rpm for 5 minutes.

7) After ~45 minutes withdraw a 5-ml aliquot from the top layer, add to a cuvette, and measure the fluorescence against a reagent blank (see manufacturer's instructions).

   NOTE: Rinse autopipet tips with ethylacetate prior to use.

Standards

Primary standard (940 ug ml^{-1}) - Dissolve 1 g of oxytetracycline-hydrochloride (Sigma Chemical) into ~900 ml DI water, and dilute to 1 liter.

Secondary standard (9.4 ug ml^{-1}) - Dilute 1.0 ml of primary standard to 100 ml.

Calculations

1) Formulate a linear equation by regressing concentations (Y-axis) against averaged fluoresence units minus the reagent blank (X-axis), and calculate the coefficient of determination (r^2).

2) Calculate sample concentrations by subtracting the blank value from the sample fluorescence, and substitute into the regression formula (Table 47).

Table 47. The relationship between fluoresence units ($\bar{\bar{fu}}$) and amounts of oxytetracycline (OTC) used to calculate a standard curve for the fluorometric analysis of OTC in fish.

Secondary standard (ml)	Total volume (L)	OTC (ug)	Fluoresence units (x)	$\bar{\bar{fu}}$ ($\bar{x}$-blank)
0.00	.015	0.00	0.00, 0.00	0.00
0.05	.015	0.47	0.36, 0.28	0.32
0.10	.015	0.94	0.91, 0.79	0.85
0.20	.015	1.88	1.60, 1.90	1.75
0.50	.015	4.70	4.02, 3.77	3.90
1.00	.015	9.40	8.72, 8.32	8.52

OTC (ug per fish) = 0.0625 + 1.1092 ($\bar{\bar{fu}}$)

$r^2 = .9979$

Anesthetizing Fish

Rearing salmonids (especially sockeye smolts) are extremely prone to handling mortality resulting from scale loss, desliming, and excitation. As both juveniles and smolts have to be captured, processed for length, weight, and implanted with coded-wire micro-tags; the fish must be sedated prior to handling.

The degree of sedation and the rates of anesthetization are controlled by concentration of either 2-phenoxyethanol or ethyl-m-aminobenzoate-methane sulfonate (MS-222). Moreover, the action of the anesthetic is slower at cooler temperatures, in weakly buffered water [alkalinity of $\leq$10 mg l^{-1} ($CaCO_3$)], and for larger fish. Consequently, preliminary tests of varying concentrations should be made against small numbers of fish to determine optimum exposure and recovery times.

Apparatus

Measuring (g) spoons, autopipets, thermometer, and immersion tubs.

Reagents

1) MS-222 - Technical grade.

2) 2-Phenoxyethanol - Reagent grade.

3) MS-222 stock solution - Dissolve ~10 g of MS-222 into 100 ml of DI water, and neutralize to pH 7 with 0.1 N NaOH.

Procedure

1) Dissolve or dilute anesthetic (Table 48) in lake water as required, immerse the fish(s), and anesthetize (~2-4 minutes).

2) Process the fish(s) immediately after sedation, revive by immersing (5-15 minutes) in clean aerated water, and release to the system.

Cautions

1) As MS-222 is a skin irritant and can cause disturbances of the central nervous system, wear rubber gloves when handling the chemical. Also, adding to poorly buffered [alkalinity of $\leq$10 mg L^{-1} ($CaCO_3$)], low pH (4.5-5.5) lake water further lowers the pH. This can be avoided by preparing a fresh stock solution, neutralizing, and dispensing as a liquid. This also reduces in-field problems associated with dissolving MS-222 at lower water temperatures. Finally, the U. S. Food and Drug Administration (FDA) has approved MS-222 for use with food fishes.

2) 2-phenoxyethanol does not lower the pH of the anesthetizing water, but does not readily dissolve in cooler (~4 C) water; however, the anesthetic will dissolve with vigorous stirring. Finally, the FDA has not approved 2-phenoxyethanol for use with food fishes, i.e., fish that are potentially consumed by humans.

Table 48. Anesthetics and generalized amounts used to prepare immersion solutions to sedate juvenile and adult salmonids.

Anesthetic	Temperature (C)	Temperature (F)	Amount (per liter)	Amount (per gal)
1) Ethyl-m-aminobenzoate-methane sulfonate (MS-222)	7-12	46-54	0.10 g	0.40 g
2) MS-222 stock solution	4-12	39-54	1 ml	4 ml
3) 2-phenoxyethanol	4-15	39-59	0.4 ml	1.5 ml

Stomach Content Analysis

Stomach contents are used to compare forage selected by fish to that available in the natural habitat. This comparison is used to assess the cause-and-effect relationship between predator and prey. Rearing salmonid juveniles are obligate (sockeye) and facultative (coho, trout) planktivores having differing abilities to forage on zooplankters. In general, facultative fishes choose larger-sized zooplankters ($\geq$1.00 mm), and, if not present, move to other food sources. In contrast, obligate planktivores have refined the ability to exploit limnetic forage and can feed on forms as small as 0.4 mm. Thus, actual prey (zooplankton) body-sizes as well as those of non-prey are used to evaluate size-selective feeding. As fishery enhancement projects seek to increase either the production of potential prey items (nutrient enrichment) or the number of predators (lake stocking); the success of enhancement depends on defining the linkage between predator and prey.

Many methods of removing stomach contents have been designed and tested; however, the preferred method is gastric lavage. Extraction efficiencies and survivals are >90%, and the technique is easy to conduct in the field by one person. The technique is similar to that described by Light et al. (1983) which uses a pressurized flow of water from a Manostat syringe to remove stomach contents.

Apparatus

10-ml Manostat syringe with 2.5-3.5-cm long piece of 1.0-mm blunted teflon tubing, 50-ml plastic centrifuge tubes with caps, funnel, wash bottle, fine tipped wash bottle, and 20-ml polyethylene scintillation vials.

Bausch and Lomb stereo dissecting microscope, 1-ml Hensen-Stemple pipet, 1-ml Sedgewick-Rafter counting cell, 0.0 to 2.0-mm ocular micrometer, and zooplankter and aquatic insect taxanomic keys (see References).

Reagents

1) Anesthetic - See Anethetizing Fish (p. 168).

2) 15% Neutralized formalin - Dilute 250 ml of 37% formaldehyde to 1 liter with tap water.

A. Gastric Lavage

Procedure

1) Anesthetize fish with either MS-222 or 2-phenoxyethanol before processing.

2) Hold the fish over a funnel attached to a sample vial, and carefully insert the teflon tubing into the stomach until resistance is felt (Figure 28).

3) Flush the stomach contents using two 5-ml injections of water from the Manostat syringe into the sample vial.

4) Measure, weigh, and then revive the fish.

5) Add neutralized formalin to the sample vial to make a 15% solution (Sturgess and Nicola 1975).

B. Stomach Removal

Procedure

1) Narcotize or kill the fish in a solution of MS-222 (~150 mg/liter) or 2-phenoxyethanol (~3 ml/liter) to prevent regurgitation of the stomach contents, and then store whole in 15% neutralized formalin.

2) Dissect the stomachs from the fish, and preserve in individual vials.

3) Label each vial with appropriate age, length, and catch data.

4) Open the stomach, and carefully wash the contents into a small petri-dish using a fine tipped wash bottle.

5) Rinse the contents into the labeled vial, and add 15% neutralized formalin.

NOTE: We have determined lavage efficiencies (E) of ~90%, for coho or sockeye juveniles, using the formula:

$$E\ (\%) = \frac{La}{La + LaD} \times 100$$

La = Number of items removed from lavaged stomachs.
LaD = Number of items removed from lavaged and dissected stomachs.

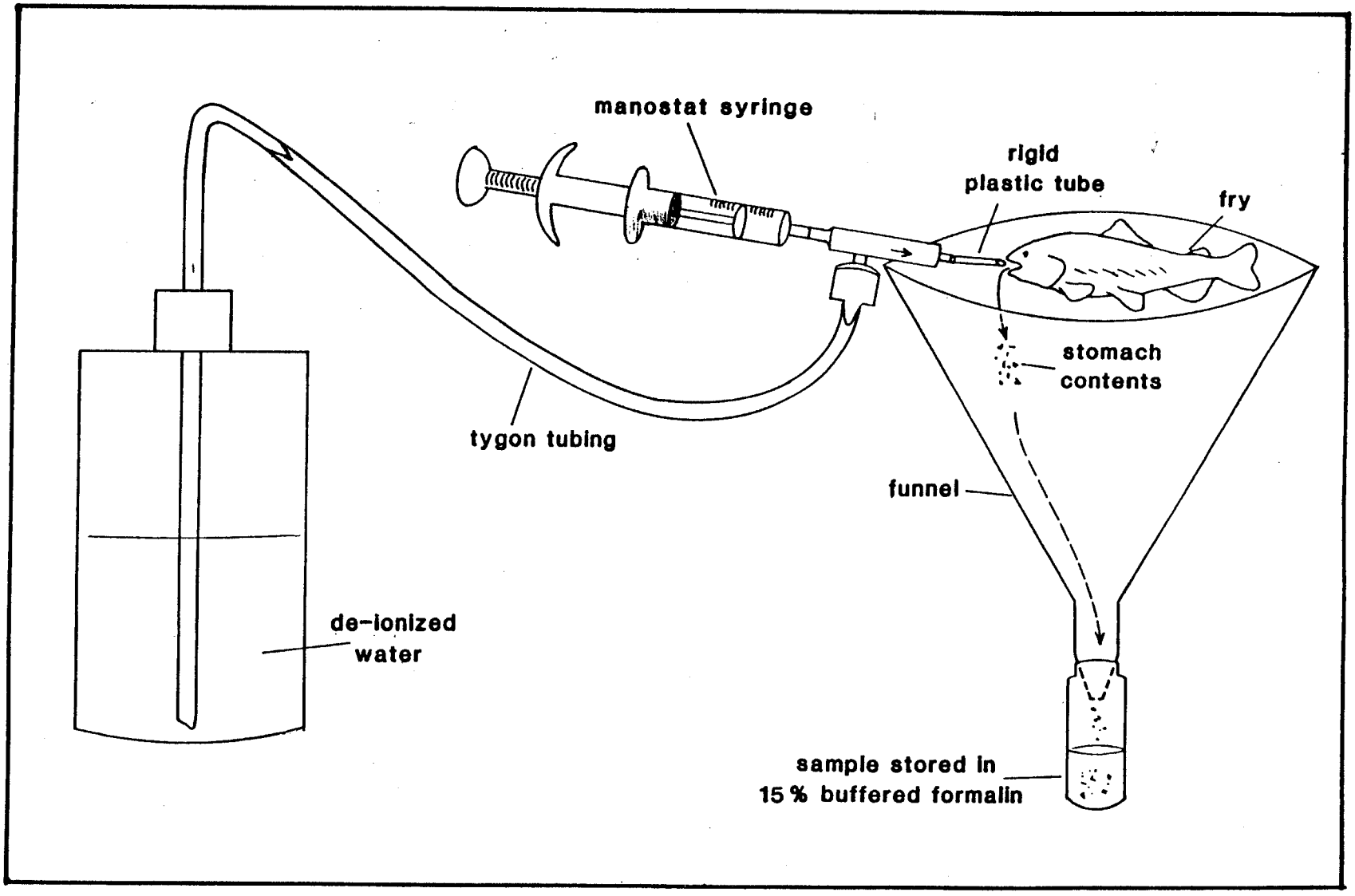

Figure 28. Manostat syringe apparatus, with teflon tube, used to remove stomach contents from juvenile salmonids by gastric lavage.

C. Identification and Enumeration

Procedure

1) Identify and enumerate zooplankters (see Volumetric and Areal Density Estimates, steps 1-7, p. 153).

2) Identify insects to genus, and count as whole organisms only distinguishable parts, e.g., head capsule.

3) Measure zooplankter body-size [see Body-Size (length) measurements, p. 155].

Calculations

1) Average the number of each zooplankter in the three 1-ml subsamples, and multiply by the total diluted volume (ml) to obtain the total number of each zooplankter consumed.

2) Sum to obtain the total number of zooplankton found in each stomach.

3) Sum the counts for each insect taxa found to obtain the total number of insects in each stomach.

4) Determine the number of fish containing forage and divide by the total number of fish sampled.

5) Determine percent incidence of each item by dividing the number of fish containing each item by the total number of fish sampled (x 100).

6) Determine percent numerical composition by dividing the number of each item by the total number of items (x 100).

Electivity Index

The electivity index (Ivlev 1961) has a range of -1 to +1; negative values indicate either avoidance or inaccessibility of a prey item, zero indicates random selection, and positive values indicate active selection. There are variations of the electivity index that compensate for bias introduced when either the abundance of prey in the environment differs substantially from prey found in the fish or when predator-prey habitats differ (Paloheimo 1979; Strauss 1979). Regardless of the version, selectivity or prey preference based on the electivity index is a relative measure until other phenomena such as probability of prey capture, and distribution of prey are better understood.

Procedure

1) Capture rearing juveniles ($\geq$30) using tow nets (Gjernes 1979), and minnow traps or beach seines for sockeye and coho respectively.

2) Remove stomach contents, identify, and enumerate (see Stomach Content Analysis, p. 170).

3) Collect zooplankters using vertical paired tows (see Plankton, p. 10), identify, and enumerate (see Zooplankter Identification, p. 152).

NOTE: Rearing juveniles and zooplankton must be from concurrent samples and habitats.

Calculations

The electivity index (E) or the degree of selection of a particular prey item by a predator is defined as:

$$E = \frac{(r_i - p_i)}{(r_i + p_i)},$$

where r_i represents the relative abundance of prey item i in the stomach of the predator expressed as a proportion or percentage of the total stomach contents, and p_i represents the relative abundance of the same prey item in the environment expressed as a proportion or percentage of the total density (Figure 29).

Diet Overlap

Considerable interest exists among biologists in the measurement of resource overlap among co-existing species e.g., sockeye salmon juveniles and stickleback. The measurements obtained can be, and have been, of more than academic interest. Unfortunately, they have also been used in the mistaken belief that the degree of overlap between the diets of two species can be directly used to assess competition. However, simply demonstrating an overlap in resource use by two species in nature can be evidence either for or against inter-specific competition. Two definitions are important:

> Overlap is the use, typically at the same time, by more than one organism of the same resource regardless of resource abundance.

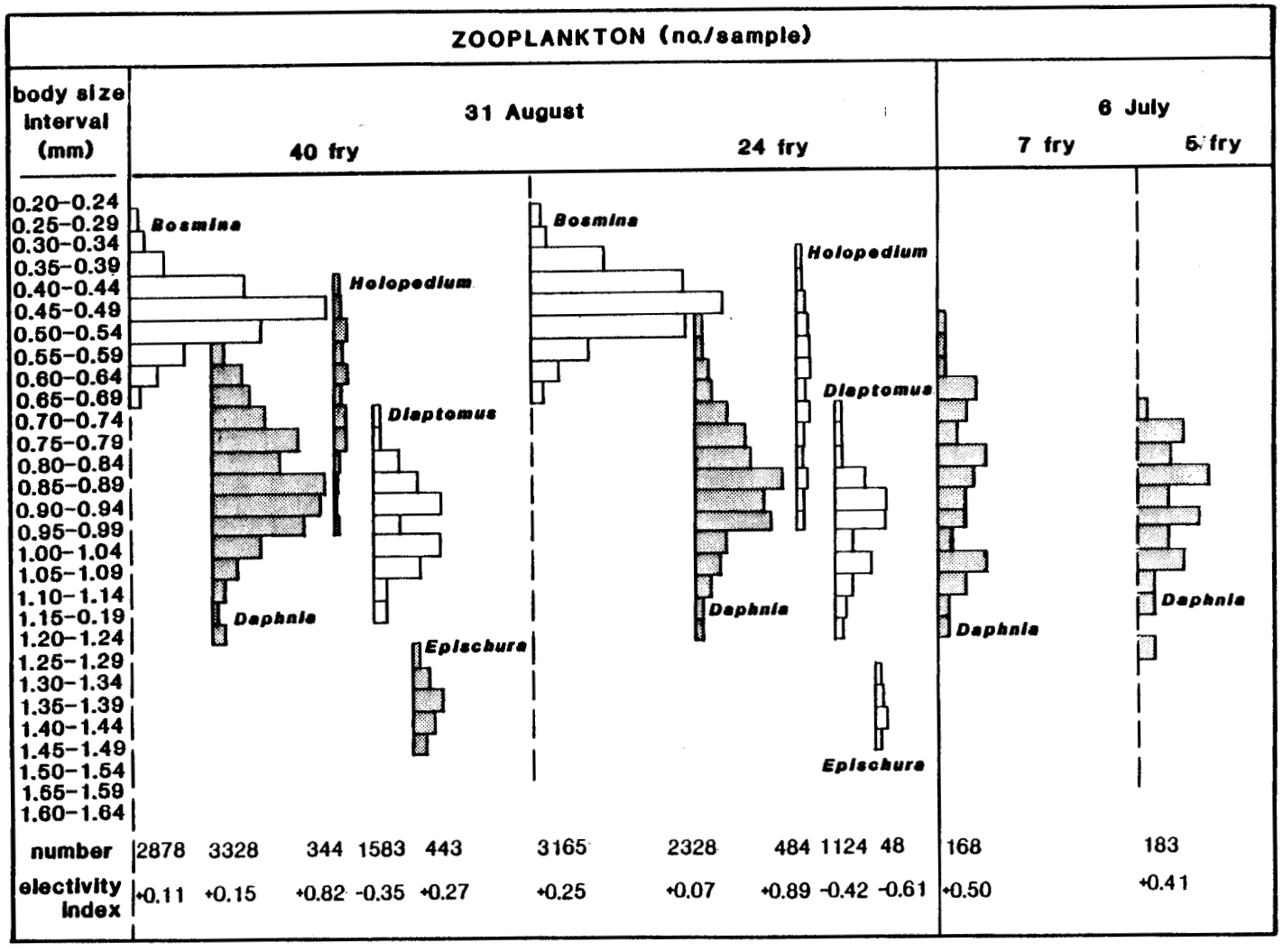

Figure 29. Range of zooplankter body-sizes from 0.2-1.64 mm (by 0.04 mm intervals) showing the length interval of each species selected by limnetic rearing sockeye fry from Packers Lake. Also shown are the total number of each zooplankter consumed, and the overall electivity index for each species.

> Competition is the demand, typically at the same time, by more than one organism for the same resource in excess of immediate supply.

Thus, to show inter- or intra-specific competition for a resource, decreased growth or survivorship, of one or both species is necessary in addition to diet overlap. In other words, if overlap in diet is high fish can be using the same resource, but are not 'limited' by such overlap as food is not of 'limiting' supply.

Procedure

1) Capture rearing juveniles ($\geq$30) using tow nets (Gjernes 1979), and minnow traps or beach seines for sockeye and coho respectively.

2) Remove stomach contents, identify, and enumerate (see Stomach Content Analysis, p. 170).

3) Collect zooplankters using vertical paired tows (see Plankton, p. 10), identify, and enumerate (see Zooplankter Identification, p. 152).

NOTE: Rearing juveniles and zooplankton must be from concurrent samples and habitats.

Calculations

Using the terminology from Morisita (1959) and Horn (1966), we have characterized subsamples x_0 and y_0 from populations X and Y respectively. Out of a total number of food categories from both samples, species i is represented x_i times in X_0 and y_i times in Y_0. If sample sizes of X and Y are relatively equal then the diet overlap coefficient (C_f) equals:

$$C_f = \frac{2 \sum_{i=1}^{n} (X_i \ Y_i)}{\sum_{i=1}^{n} (X_i^2) + \sum_{i=1}^{n} (Y_i^2)}$$

This formula is also appropriate where the data are expressed as the proportions x_i and y_i of the respective samples composed of species i. This empirical measure has an upper limit of exactly 1, and ranges between 0 (no overlap) to 1 (complete overlap). Zaret and Rand (1971) considered a $C_f \geq 0.60$ to indicate 'significant' overlap.

Example

Samples were taken, by tow net, of the limnetic rearing juveniles in Fred Lake, and stomach contents removed by gastric lavage. Our subsamples of the rearing fishes came from the same habitat and were taken at the same time. We compared the diet of sockeye juveniles and threespine stickleback by calculating the diet overlap coefficient (C_f) using food type proportions (Table 49).

Table 49. The proportions of food items (i) found in sockeye juveniles (Y) and threespine stickleback (X) used to calculate the diet overlap coefficient (C_f).

Food Item (i)	Fish group X			Fish group Y			
		i	i^2		i	i^2	$x_i\ y_i$
Daphnia	x_1	0.49	0.240	y_1	0.25	0.063	0.123
Bosmina	x_2	0.25	0.063	y_2	0.10	0.010	0.025
Holopedium	x_3	0.10	0.010	y_3	0.05	0.003	0.005
Cyclops	x_4	0.10	0.010	y_4	0.49	0.240	0.049
Diaptomus	x_5	0.05	0.003	y_5	0.11	0.012	0.006
Diptera	x_6	0.01	0.000	y_6	0.00	0.000	0.000
Sum		1.00	0.326		1.00	0.328	0.208

$$C_f = \frac{2 \times 0.208}{0.326 + 0.328} = 0.64$$

Freshwater Cohort Production

Freshwater salmon production is commonly expressed as gross yield of smolt biomass (kg). However, because of differences in either stocking time, initial fish densities and mean sizes, or all three; it is useful to present the yield of smolts as a net change in biomass over time (t). That is, introduced (fry plants) or initial in-lake biomass (estimated by hydroacoustic techniques or by mark-recapture) subtracted from the gross yield of smolts equals net biomass produced. The net change in biomass (kg or kg/ha) allows comparisons of freshwater salmon production between lakes, and within the same lake but between years.

Methods used to estimate cohort prc
numbers (N_t) and individual weights (
stages. Allan (1951) graphically descr
from a curve relating the number of
individual weights. The area beneath
using planimetry and can be used to
production. Alternately, Gillespie and
production equations based on the Allen
be used to calculate cohort production.

Procedure

1) Estimate the number of juveniles t
estimate in-lake populations (N) b
mark-recapture, and the mean indivi
and Koenings 1985).

2) Estimate the number of in-lake juv
of the growing season (October to
geographical location) hydroa
mark-recapture, and the mean indivi
and Koenings 1985).

3) Determine the number of smolts
individual weight (W_3) (Blackett et
Lebida 1984, Flagg 1985, and Koenin

Calculations

1) Construct a plot of numbers of
against mean individual weight (X-a

2) Determine, with a calibrated plan
Calibrations, p. 192), the individu
X, Y, and Z (Figure 30), used to
parameters (Table 50).

3) Alternately, calculate the areas of s
Z (Figure 30), and determine the p
(Table 50).

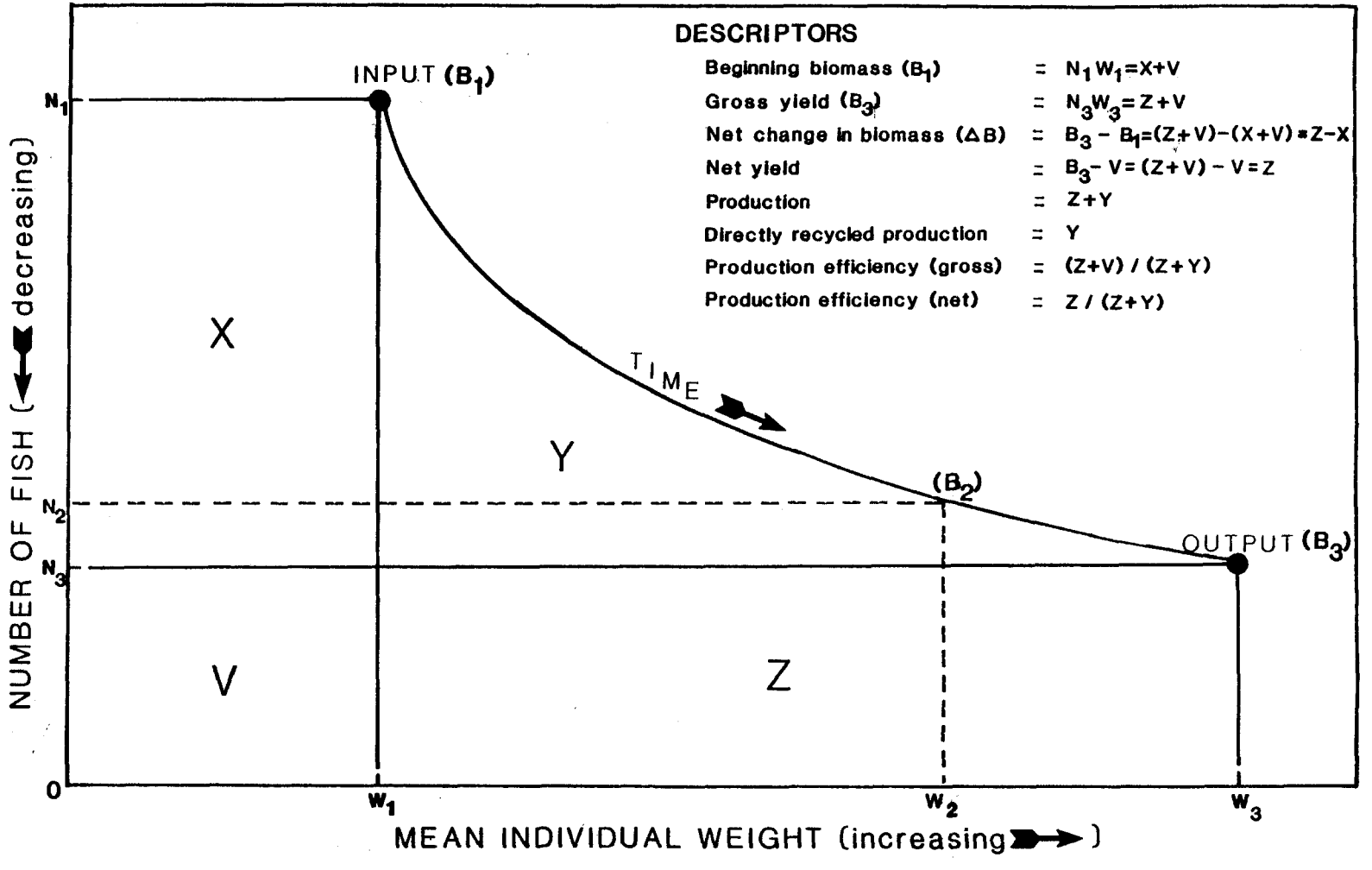

Figure 30. Generalized Allen curve showing the relationship between the number of fish and mean individual weight used to calculate production parameters (see descriptors) for rearing fish. Biomass (B) estimates shown represent the life history stages of spring juveniles (B_1), late fall juveniles (B_2), and smolts (B_3).

Table 50. Summary of cohort production parameters described by Allen (1951) and determined either by planimetry or from equivalent equations following Gillespie and Benke (1979).

Planimetered area	Equivalent calculation	Production parameters
X + V	$N_1 \times W_1$	Initial biomass (B_1)
Z + V	$N_3 \times W_3$	Gross yield (B_3)
Z	$W_3 - W_1 \times N_3$	Net yield
Y	$\frac{(W_3 - W_1)(N_1 - N_3)}{2}$	Recycled production
Z + Y	Net yield + recycled production	Total production
(Z+V)/(Z+Y)	Gross yield/total production	Gross production efficiency
Z/(Z+Y)	Net yield/ total production	Net production efficiency

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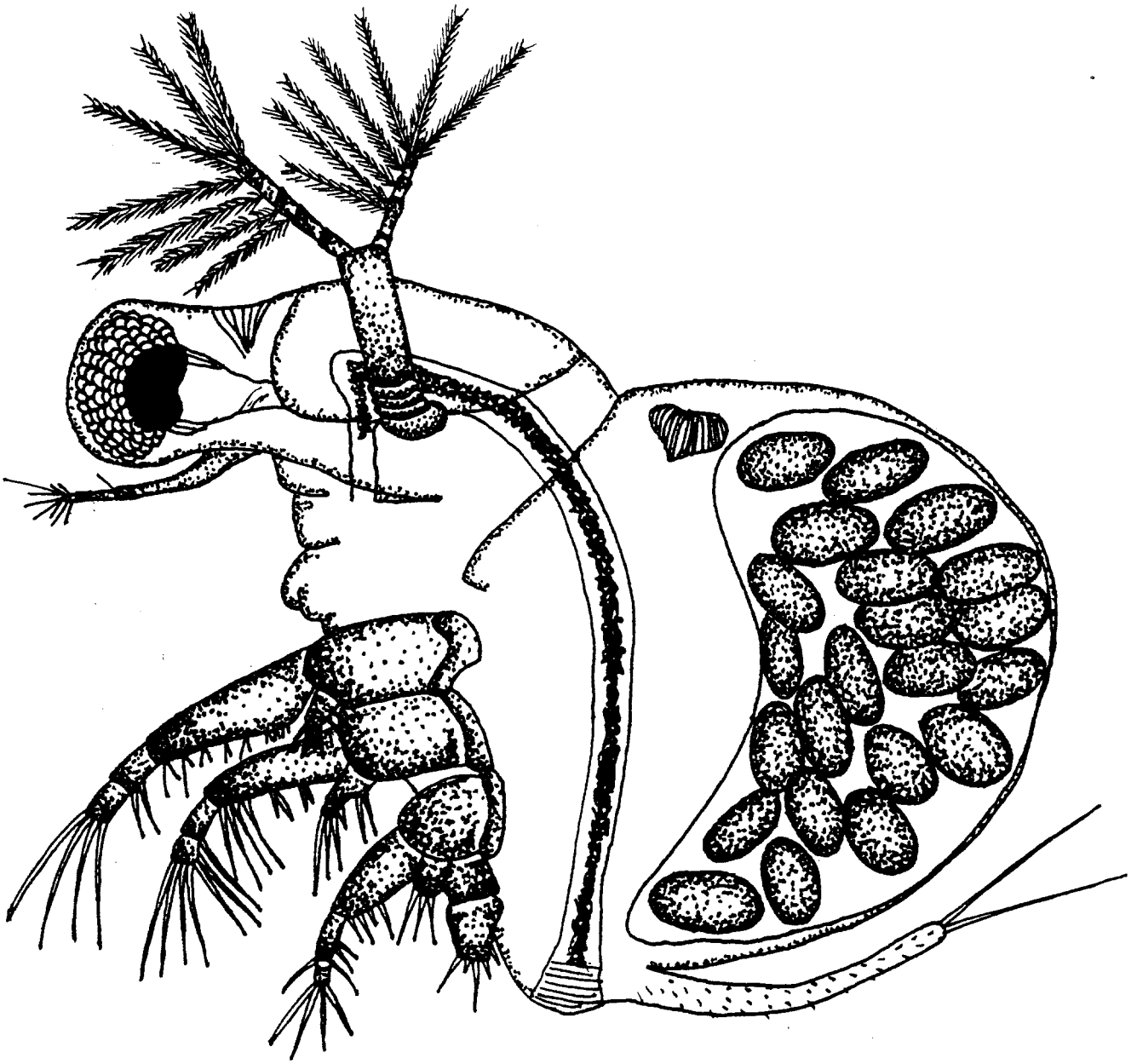
PART III.

QUALITY ASSURANCE, CALIBRATIONS, AND STATISTICAL EVALUATION



QUALITY ASSURANCE

Laboratory Technique

Quality assurance in the limnology laboratory is achieved by 1) use of highly purified (Type I) water, 2) properly cleaned glassware, 3) use of reagent-grade chemicals, 4) frequent maintenance and calibration of instruments, 5) daily preparation of standards, and 6) replicated standard and sample analysis. While no single factor determines precise and accurate results, each adds a fundamental part to internal quality control.

To assure precision and accuracy of analytical results, it is crucial that the laboratory have a source of water free of contaminants for preparing standards and reagents, and for washing glassware. Use of Type I water is the most significant factor affecting methodological capabilities and is especially critical when analyzing for dilute concentrations. Thus, laboratory water is monitored daily for purity, and appropriate maintenance measures taken when water quality falls below Tyle I standards.

Laboratory analysis procedures require glassware and plasticware for preparing and transferring standards, reagents, and samples. To avoid contamination it is essential to adhere to the cleaning procedures outlined in Part I (p. 6) to assure consistent and accurate analytical results.

Chemicals and reagents are manufactured with different levels of purity. That is, product containers with the same chemical name or formula may not be of similar quality. Use only substances certified by the American Chemical Society and of analytical-reagent grade. Use of more inferior grades e.g., a technical quality, may result in a reduction of analytical accuracy.

Laboratory instruments are generally regarded as being reliable and stable; however, minor adjustments of components, cleaning, and carefully conducted calibrations with known reference materials are routinely required. Specific calibration procedures are detailed in the section: Instrument Calibrations (p. 192).

Analysis of standards is used to assess levels of precision and accuracy of methodologies. Calibration standards are prepared by adding known quantities of pure chemical to DI water, and then analyzed under the same conditions as the samples. The results are used to formulate a calibration curve to determine sample concentrations. By comparing calibration curves it is possible to detect error and biases in the methodology.

Systematic and random error are minimized by using a calibration curve when analyzing each test lot.

For each analysis, two aliquots of sample and standard are analyzed and the mean result reported as the best estimate of the constituent; however, a third aliquot is analyzed and averaged in place of a biased or erred value.

While the laboratory techniques described above are key to quality assurance, it is essential that analytical results be confirmed by an independent testing agency. Confirmation provides confidence and reliability in analytical results and more importantly, allows data to be comparable with that of other laboratories. The limnology laboratory utilizes several outside agencies for confirmation of internal quality control.

General Tests and Nutrients

Each year the limnology laboratory participates in the U. S. Geological Survey (USGS) Standard Reference Water Sample Program which distributes reference samples to laboratories nationwide. In 1985, over 100 laboratories participated thus, providing a broad database to evaluate laboratory capabilities and performance. Analytical results are submitted to the USGS which list the results from each laboratory, provides a statistical evaluation, and scores the performance for each reference value as follows:

Performance Rating	Standard Deviations from the mean value
4 (excellent)	0.00 - 0.50
3 (good)	0.51 - 1.00
2 (satisfactory)	1.01 - 1.50
1 (questionable)	1.51 - 2.00
0 (poor)	>2.00

USGS reference values as determined by participating laboratories, the values reported by the limnology laboratory, and the performance rating for each parameter are summarized in (Tables 51-54). Nutrients and metals are reported in mg L^{-1}, pH in units, and conductivity in umhos/cm. Finally, turbidity determinations are referenced to the U. S. Environmental Protection Agency (EPA) Environmental Monitoring and Support Laboratory (Cincinnati, Ohio) standards.

Table 51. Results from the 1982 U. S. Geological Survey Standard Water Reference Sample Program showing the constituents analyzed in three reference samples, mean values, and values determined by the limnology laboratory with performance ratings.

Sample	Value determined by the limnology laboratory	Mean value of all laboratories	Rating
P4			
Conductivity	8.9	9.25	4
pH	6.05	6.20	4
Calcium	1.5	0.8	0
Magnesium	<0.3	0.121	-
Nitrate	0.123	0.122	4
Total ammonia	0.002	0.054	3
N-15			
Reactive phosphorus	1.2	1.08	4
Total phosphorus	1.8	1.68	4
Organic nitrogen	0.4	0.93	1
Nitrate	4.3	4.28	4
Nitrite	0.21	0.20	4
Total ammonia	0.79	0.365	1
M-84			
Conductivity	1090	1089	4
pH	8.21	8.90	3
Alkalinity	167	161	2
Calcium	97	87	1
Magnesium	44	52	0
Silica	7.7	7.59	-
Total phosphorus	0.7	0.65	3
Nitrate	3.7	3.94	3
Nitrite	0.46	0.459	4

Table 52. Results from the 1983 U. S. Geological Survey Standard Water Reference Sample program showing the constituents analyzed in four samples, mean values, and value determined by the limnology laboratory with performance ratings.

Sample	Value determined by the limnology laboratory	Mean Value of all laboratories	Rating
P-5			
Conductivity	4.2	4.278	4
pH	5.63	5.829	4
Calcium	0.4	0.29	2
Magnesium	<0.5	0.32	-
Total ammonia	0.002	0.022	3
Nitrate	0.08	0.084	4
M-86			
Conducitivity	839	859	4
pH	8.1	8.13	4
Alkalinity	150	150.7	4
Calcium	65	70.6	3
Magnesium	22	28	0
Silica	13.2	12.62	4
Total phosphorus	0.54	0.497	3
Nitrite	0.01	0.011	4
Nitrate	4.61	3.975	1
N-10			
Total phosphours	1.58	1.432	3
Reactive phosphours	0.98	1.00	4
Organic nitrogen	0.51	1.342	3
Total ammonia	1.53	1.191	2
Nitrate	2.55	2.478	3
Nitrite	0.004	0.008	3
N-11			
Total phosphorus	0.54	0.513	4
Reactive phosphorus	0.49	0.487	4
Organic nitrogen	0.18	0.732	2
Total ammonia	0.29	0.16	1
Nitrate	4.14	3.962	3
Nitrite	0.013	0.014	4

Table 53. Results from the 1984 U. S. Geological Survey Standard Water Reference Sample Program showing the constituents analyzed in three samples, mean values, and values determined by the limnology laboratory with performance ratings.

Sample	Value determined by the limnology laboratory	Mean value of all laboratories	Rating
N-13			
Ammonia	0.90	0.19	4
Nitrite	0.03	0.02	2
Nitrate	0.20	0.23	4
Organic nitrogen	0.10	0.26	3
Total phosphorus	0.10	0.09	4
Reactive phosphorus	0.10	0.09	3
P-7			
Conductivity	8.8	9.96	3
pH	6.00	6.42	2
Calcium	1.05	0.91	3
Magnesium	<0.34	0.17	0
Ammonia	0.01	0.03	3
Nitrate	0.05	0.06	3
M-90			
Alkalinity	0.00	3.2	2
Calcium	18.00	25.3	0
Magnesium	9.00	10.4	1
Nitrite	0.01	0.013	4
Nitrate	0.20	0.26	3
Total phosphorus	0.00	0.02	3
Silica	12.00	13.20	2

Table 54. Results from the 1985 U. S. Geological Survey Standard Water Reference Sample Program showing the constituents analyzed in two samples, mean values, and values determined by the limnology laboratory with performance ratings.

Sample	Value determined by the limnology laboratory	Mean value of all laboratories	Rating
M-14			
Conductivity	110	111.2	4
pH	7.31	7.488	4
Alkalinity	28.0	27.2	4
Calcium	11.2	11.4	4
Magnesium	2.9	2.95	4
Nitrite	<0.00	0.005	0
Nitrate	0.188	0.18	4
Total phosphorus	0.019	0.01	3
Silica	7.18		3
N-15			
Ammonia	0.55	0.56	4
Nitrite	0.00	0.043	0
Nitrate	0.96	0.953	4
Organic nitrogen	0.738	0.45	3
Total phosphorus	0.77	0.782	4
Reactive phosphorus	0.191	0.19	4

Primary Productivity/Production

Carbon-14 (C-14) isotopes are supplied by ICN Pharmaceuticals, Irving, California and stipulated to have a true activity between the limits of ±5% at the 95% confidence level. Liquid scintillation spectrometer calibration is based on the efficiency of detecting Beta-particle emission of a C-14 standard prepared by New England Nuclear, Boston, Massachusettes. We have consistently detected activity or disintegrations per minute (DPM) within ±5% of the known reference.

Algal standing crop measurements are referenced to chlorophyll *a* (chl *a*) and phaeophytin *a* (phaeo *a*) standards of algal extracts provided by the EPA (Table 55). In addition, the standards are used for both the spectrophotometric and fluorometric procedures and for calibrating the filter

fluorometer. As EPA reference standards may not be available, chl a and phaeo a extracts obtained from Sigma Chemical Company, St. Louis, Missouri can be used to prepare standards. Finally, algal enumeration and identification is conducted using phase microscopy at magnifications of 450x and 1000x by Eco-Logic Ltd., West Vancouver, British Columbia.

Table 55. Comparisons of chl a and phaeo a levels in reference standards of the U. S. Environmental Protection Agency, as determined using the spectrophotometric and fluorometric procedures by the limnology laboratory.

Constituent	Method	EPA reference value ($ug\ L^{-1}$)	Confidence limits ($ug\ L^{-1}$)	Limnology laboratory value ($ug\ L^{-1}$)
Chl a	Fluorometric	3.2	2.4 - 4.0	3.7
Phaeo a	Fluorometric	1.4	0.8 - 2.0	1.9
Chl a	Fluorometric	16.7	14.2 -19.2	18.0
Phaeo a	Fluorometric	-0.1	-2.4 - 2.1	2.1
Chl a	Trichromatic	7.76	7.57- 7.96	7.45
Chl a	Trichromatic	0.6	0.47- 0.74	0.74
Chl a	Trichromatic	1.29	1.14- 1.44	1.18
Chl a	Monochromatic	6.29	5.85- 6.73	5.95
Phaeo a	Monochromatic	2.11	1.34- 2.88	2.19

Zooplankton

Species identification is determined through the use of accepted taxanomic keys as listed in the methodology. In addition, reference slides are prepared of each species and submitted for verification by Thomas E. Bowman, Director of Crustacea, at the Smithsonian Institute, Washington, D. C.

INSTRUMENT CALIBRATIONS

Proper use, maintenance, and calibration of laboratory instruments is critical to the precision and accuracy of analytical results. Equipment must be cleaned, adjusted and properly calibrated against quality reference standards at intervals specified in the methods or as recommended by the manufacturer.

Dissolved Oxygen/Temperature Meter

Inspect the probe membrane for any air bubbles, holes or creases, and replace if necessary. It is important to calibrate the meter at each sampling station to insure accurate D.O. measurements. The preferred calibration technique is to compare the meter reading (D.O. probe at subsurface depth) with the D.O. value of the subsurface depth determined by the Winkler method. Air calibration should be used only if recommended by the manufacturer. Calibration uses a Hach Chemical D.O. kit for the method outlined below.

1) Fill a 60-ml BOD bottle and cap making sure no air bubbles are trapped inside.

2) Add 1 pillow of D.O. I reagent and shake well.

3) Add 1 pillow of D.O. II reagent and shake. Allow the floc to settle until the upper half of the solution is clear. Mix again and let settle.

4) Add 1 pillow of D.O. III reagent and dissolve. The solution will turn a clear yellow or gold in the presence of oxygen.

5) Add a few drops of standard (2%) starch solution. The sample will turn blue-black.

6) Divide the sample and titrate each 30-ml subsample with 0.019 N phenylarsine oxide (PAO) using a 5.0-ml syringe. The solution will become clear when the endpoint is reached.

7) Calculate the D.O. from the appropriate expression:

   $mg\ L^{-1}$ D.O. = ml titrant x 5.81, for a 15-ml sample.

   $mg\ L^{-1}$ D.O. = ml titrant x 2.9 for a 30-ml sample.

   Average the results of the 2 subsamples.

8) Immerse the D.O. probe and set the meter reading to the value obtained from the Winkler titration.

Spectrophotometer

Throughout the manual the term absorbance (abs), synonymous with optical density or light extinction, is used as the measure of color intensity in spectrophotometric determinations. Absorbance measurements can also be converted to percent transmission (T) using:

$$\log T = 2 - abs \quad \text{or} \quad abs = -\log (T \times .01)$$

Spectrophotometers are calibrated using the procedure described below:

1) Allow the instrument to warm up for 15 minutes, select the wavelength specified in the method, and switch to absorbance mode.

2) Clean two quartz-glass optical cuvettes (capable of transmission at wavelengths of 200-900 nm), fill with DI water, and place in the reference and sample compartments.

3) Adjust the blanking control until the reference cuvette reads .000 absorbance and, without changing the control, measure the absorbance of the sample cuvette. If the absorbance differs by a reading of $\pm$ .003, the cuvettes are considered optically matched and can be used to read sample and standard absorbances.

5) Read standards and sample absorbances, and rezero the cuvette as necessary between samples using the blanking control.

NOTE: Matched cuvettes for one wavelength does not assure they are optically matched at a different wavelength. Therefore, calibrate and rezero the instrument each time the wavelength is changed.

Autoanalyzer

1) Pump DI water through the system for 10 minutes.

2) Set the toggle switch inside the colorimeter to 'D' (direct chemistry). Turn the rotary switch to 'zero' and to 'full scale' making sure the recorder pen responds, and adjust the set screws if necessary.

3) Set the standard calibration control to 1.0 and the baseline control midway. Establish a water baseline by opening (clockwise rotation) both apertures and if the pen is below zero, slowly rotate aperature A counterclockwise until the pen is at zero. If the pen is above zero, slowly rotate B counterclockwise to adjust.

4) Place the reagent feed lines in their containers and pump through the system for 10 minutes, and note the reagent deflection line. An abnormal deflection line indicates either one or more reagents or pump tubes need replacing. Zero using the baseline control if the deflection line is comparable to previous reagent calibrations.

5) Pump the highest standard through the system. When the probe returns to the sample wash, pump for 5 minutes, and repeat twice. As the highest standard appears on the chart recorder, rotate the standard calibration control to maximum chart deflection. Lock the control and record the standard calibration value.

6) Pump the standards through the system from lowest to highest concentration, and return the probe to the sample wash for 2 minutes to avoid contamination of the first sample.

7) Run the samples, and following completion, place the reagent lines in the system wash or DI water, and continue operation for 15 minutes. Remove the pump platen to prolong pump-tube life.

Filter Fluorometer

Calibration procedures using known chl a standards should be conducted quarterly, following a lamp change, and after adjusting the attenuator.

Standards

Primary standard - Dissolve ~1 mg of pure chl a (Sigma Chemical Company, St. Louis, MO) into ~80 ml of 90% neutralized acetone, and dilute to 100 ml with 90% acetone.

Secondary standard - Dilute 1 ml of the primary standard to 100 ml with 90% acetone.

Procedure

1) Using the spectrophotometer, measure the absorbances of the secondary standard against a 90% acetone blank at 663

nm, and calculate the chl a concentration using the monochromatic method (p. 148).

2) Prepare serial dilutions of the secondary standard with 90% acetone in 20-ml glass vials covered with aluminum foil so that fluorescence readings are between 15 and 85 units for at each sensitivity level (Table 56).

3) Pour ~4 ml of each diluted standard into separate cuvettes, and measure the fluorescence (Rb) three times during a one minute period at the appropriate sensitivity level(s).

4) Add 2 drops of 2 N HCl to each cuvette using a 0.05-ml autopipet, invert, and after 30 seconds measure the fluorescence (Ra) three times during a one minute period.

Calculations

1) Divide the averaged Rb value by the averaged Ra value for each standard read at that sensitivity level to obtain the acid ratio (rs) for each sensitivity level.

2) Divide the concentation (ug L^{-1}) of the diluted standard (Step 1) by the averaged Rb value for each concentration to obtain a conversion factor (FS) for each sensitivity level.

3) Calculate a calibration factor (Sx) for each sensitivity level using:

$$Sx = \bar{\bar{FS}} \frac{\bar{\bar{rs}}}{(rs - 1)}$$

Planimeter

The planimeter is used to integrate the area beneath a curve; however, the instrument reading is not directly equivalent to actual units of area. Therefore, the planimeter must be calibrated to convert instrument readings to measured area.

1) Place the paper with the area to be measured on a smooth flat surface, and securely fasten with tape.

2) Rule off a known area (A), carefully trace 3 times, and record each planimeter reading (P).

3) Average the readings, and determine a calibration factor (F) as:

$$F = \frac{A}{\bar{P}}$$

Table 56. **Fluorscence units before (Rb) and after (Ra) acidification used to obtain acid ratios (rs) and calibration factors (Sx) at various sensitivity levels (S) for the fluorometric analysis of chl a.**

Sensitivity level (S)	Volume of secondary standard (ml)	Total volume (L)	Standard chl a ($ug\ L^{-1}$)	$\bar{\bar{Rb}}$	$\bar{\bar{Ra}}$	rs ($\bar{\bar{Rb}}/\bar{\bar{Ra}}$)	Fs (chl a/$\bar{\bar{Rb}}$)	Sx [Fs(rs/rs-1)]
1X	15.0	.020	80.3	52.3	24.6	2.1260	1.5354	2.8990
3X	3.0	.020	16.1	53.1	26.4	2.0114	0.3032	-.6030
10X	1.0	.020	5.4	84.9	42.1	2.0166	0.0636	0.1262
30X	0.1	.020	0.5	19.1	9.4	2.0319	0.0262	0.0516

$$\text{Chl a } (ug\ L^{-1}) = Sx\ (Rb-Ra)$$

$$\text{Phaeo a } (ug\ L^{-1}) = Sx\ (rs \times Ra - Rb)$$

NOTE: Chl a and phaeo a indicated here are not in-lake concentrations.

4) Trace the unknown area three times, average P, and multiply by F to obtain the measured area.

pH Meter

1) Allow the meter to warm-up for 5 minutes while preparing the probe(s) according to the manufacturers instructions.

2) Select 2 buffers; pH 7, and a second buffer of either pH 4 or 10 to cover the range of the unknown solutions. Pour 100 ml of each into separate beakers, and measure the temperature of the solutions.

3) Place the pH 7 buffer on the magnetic stirrer, immerse the probe, and rotate the calibration control until the meter reading equals 7.0.

4) Rinse the probe with DI water, immerse into the second buffer, and rotate the temperature compensator until the meter reading equals the pH of the second buffer.

5) Rotate the slope indicator until the temperature compensator points to the temperature of the buffers and read the slope value. If the slope value is >90% the meter is calibrated. If the slope is <90% either the probe is defective or one of the buffers is contaminated.

Ocular Micrometer

The Bausch and Lomb (B&L) model 31-16-43 micrometer is calibrated with a dual-scale stage micrometer prior to measuring zooplankter body-lengths. Calibrate the ocular micrometer using the 0-2.00-mm (0.02 divisions) stage scale when measuring body-lengths $\leq$2.00 mm. Calibrate the ocular micrometer using the 0-10 mm (0.1 divisions) stage micrometer scale when measuring body-lengths >2.00 mm.

1) Insert the ocular micrometer into one of the oculars and position the stage micrometer.

2) Set the zoom control to 5X to calibrate the 0-2.00 mm scale, and 1X to calibrate the 0-10.0 mm scale. Focus on the appropriate scale until the divisions of both micrometers are superimposed.

METHODOLOGIES: INTERFERENCE AND STATISTICAL EVALUATION

Interference

Interfering substances artificially enhance or reduce the analytical level of another substance. In general, there are two types of interference, 1) turbidity which effects colorimetric methods by enhancing absorbances, and 2) chemical which affects colorimetric and titrametric methods by reducing absorbances or titrant volumes respectively. Interference can be eliminated by use of either turbidity blanks, spiking with equivalent amounts of interfering substance or sample dilutions.

Turbidity

Turbidity, due to the presence of suspended particles e.g., silt, causes increased spectrophotometric absorbances and autoanalyzer chart deflections. If left uncorrected, analytical results will be overestimated; however, corrections can be made using turbidity blanks. Turbidity blanks are prepared by substituting DI water for the indicator or color-producing reagent and then analyzed. For example, an equal volume of DI water is added in place of ammonium molybdate in the analysis of reactive silicon and total phosphorus.

To correct for turbidity, subtract the absorbances or chart deflections of both the turbidity blank and the reagent blank from the sample absorbance. Substitute the corrected value into the regression formula as described in the specific methodologies.

Chemical

Typically, interfering chemicals lower analytical results by either reacting with the constituent being analyzed forming an unreactive complex or reacting with the reagents to prevent the substance from being analyzed. For example, iron concentrations above 10 mg L^{-1} reduce color formation in the analysis of total phosphorus. One method of compensating is to add the interfering substance to the calibration standards. That is, if the concentration of the interfering substance is known, add the same amount to the standards.

Alternately, a series of sample dilutions can be made prior to the analysis to reduce the concentration of the interfering substance. If interference is occurring, diluted samples will have increased absorbances or chart deflections. To obtain the correct concentration, multiply the concentration of the diluted sample by the appropriate dilution factor (see p. 39).

Statistical Evaluation

Standard Range

The standard range is defined by the highest and lowest standard concentration routinely used to formulate a calibration curve. Preparing standards using these limits is generally sufficient to accomodate those concentrations found for Alaskan lakes. The standard range is not equivalent to the operating range which is defined by the lower and upper limits of detection (Table 57).

Upper Limit of Detection

The upper limit is the maximum concentration that the method is capable of detecting. Sample concentrations exceeding the upper limit of detection must be diluted prior to analysis.

Lower Limit of Detection

The lower limit of detection is the lowest concentration detected as significantly greater than the reference blank. Detection limits are derived empirically or statistically (Table 57). Empirical detection limits were derived by decreasing standard concentrations until absorbances, titrant volumes, and chart deflections were significantly different from the reagent blank value. The statistical or predicted limit was obtained by formulating an aggregate linear regression using the results of 30 representative calibration curves. The Y-intercept of the upper limit of the 95% confidence interval is the predicted lower limit of detection.

Precision

Precision (P) refers to the reproducibility of test results under operating conditions, and is expressed as the coefficient of variation (CV):

$$P = CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Although the level of precision may vary at different concentrations, the CV reported is for a mid-range standard concentration (Table 57).

Table 57. Statistical evaluations of analytical methodologies used by the limnology laboratory.

Methodology	Standard range	Lower limits of detection: Empirical	Lower limits of detection: Predicted	Precision	Accuracy
Calcium (titration)	2.5-12.5 mg/l	0.3 mg/l	0.20 mg/l	11% @ 5 mg/l	± 5% @ 5 mg/l
Calcium (colorimetric)	0.2- 4.0 mg/l	0.1 mg/l	0.04 mg/l	3% @ 1 mg/l	± 6% @ 1 mg/l
Magnesium (titration)	1.5- 7.5 mg/l	0.3 mg/l	0.20 mg/l	21% @ 3 mg/l	± 11% @ 3 mg/l
Magnesium (colorimetric)	0.4- 4.0 mg/l	0.4 mg/l	0.24 mg/l	24% @ 1 mg/l	± 20% @ 1 mg/l
Total iron (manual)	10- 200 ug/l	4.0 ug/l	3.30 ug/l	17% @ 40 ug/l	± 7% @ 40 ug/l
Total iron (automated)	5- 500 ug/l	2.0 ug/l	N.A.	N.A.	N.A.
Reactive silicon (manual)	100-4000 ug/l	20.0 ug/l	27.00 ug/l	6% @ 1868 ug/l	± 2% @ 1868 ug/l
Reactive silicon (automated)	100-1750 ug/l	20.0 ug/l	33.00 ug/l	17% @ 583 ug/l	± 2% @ 583 ug/l
Kjeldahl nitrogen (automated)	50- 300 ug/l	3.0 ug/l	3.50 ug/l	8% @ 100 ug/l	± 3% @ 100 ug/l
Total ammonia (manual)	1- 50 ug/l	1.0 ug/l	0.25 ug/l	15% @ 15 ug/l	± 8% @ 15 ug/l
Total ammonia (automated)	12.5- 100 ug/l	1.0 ug/l	1.10 ug/l	13% @ 20 ug/l	± 7% @ 20 ug/l
Nitrate + nitrite (manual)	2- 200 ug/l	1.0 ug/l	0.60 ug/l	7% @ 50 ug/l	± 2% @ 50 ug/l
Nitrate + nitrite (automated)	20.0- 100 ug/l	1.0 ug/l	3.40 ug/l	9% @ 50 ug/l	± 3% @ 50 ug/l
Total phosphorus (manual)	2- 20 ug/l	0.5 ug/l	0.25 ug/l	5% @ 6 ug/l	± 3% @ 6 ug/l
Total phosphorus (automated)	2.5- 50 ug/l	1.0 ug/l	1.20 ug/l	12% @ 13 ug/l	± 9% @ 13 ug/l
Reactive phosphorus (manual)	1- 10 ug/l	0.5 ug/l	0.12 ug/l	7% @ 4 ug/l	± 4% @ 4 ug/l
Organic carbon (manual)	60- 600 ug	30.0 ug	24.00 ug	25% @ 300 ug	± 9% @ 300 ug
Total particulate phosphorus (manual)	1- 14 ug	0.05 ug	0.02 ug	3% @ 5 ug	± 3% @ 5 ug
Total particulate phosphorus (automated)	1- 11 ug	0.60 ug	0.40 ug	6% @ 5 ug	± 7% @ 5 ug
Inorganic particulate phosphorus	1- 7 ug	0.05 ug	0.08 ug	12% @ 2.25 ug	± 3% @ 2.25 ug
Organic particulate phosphorus	1- 14 ug	0.05 ug	0.03 ug	10% @ 5 ug	± 4% @ 5 ug

N.A. = Not available

Accuracy

Accuracy (A) is defined as the difference between the analytical result and the true value. That is, the difference between a prepared standard (true value), and the result of the analysis (observed) (Table 57). Thirty determinations were made, the mean absolute deviate calculated, and expressed as a percent of the true value:

$$A = |\text{observed} - \text{true value}|/\text{true value} \times 100$$

Procedures

Levels of precision and accuracy permit acceptance or rejection of analytical results. In general, analytical data are accepted if error, measured as accuracy, is of the order of $\pm$10%. We have statistically determined the accuracy of our methodologies to be $<\pm$10% with the exception of the magnesium analysis (Table 57). The methodological capabilties and their analytical limits were derived from thirty individual calibration curves for each procedure, and were carried out by several technicians over a period of two years. As such, the results of the statistical evaluations (Table 57) represent realistic limits for routine testing, and do not result from 'specialized' procedures. Consequently, the analytical determinations using our methodologies can be expected to yield results within the given statistical limits.

Calculations

Colorimetric analyses are based on the principle of Beer's law which states that optical density (absorbance) at a specific wavelength can be correlated to the concentration of a specific substance. In addition, titrant volumes can also be correlated to concentrations. Standards are analyzed, and results used to formulate a linear equation by regressing concentrations against the color intensity (absorbances or chart deflections), and titrant volumes. These types of correlation are mathematically derived using linear regression (Figure 31).

Linear regression is determined by the method of least squares, which minimizes $(Y - \hat{Y})^2$. The slope of the regression (m) refers to the steepness of the line, and may be either positive, describing an increase in absorbance with concentration (Figure 31A) or negative, describing a decrease in absorbance with concentration (Figure 31B). The Y-intercept is the intersection of the regression line on the Y-axis, and can be positive, negative, or zero. Thus, the regression line is mathematically described by the slope and the Y-intercept.

The coefficient of determination (r^2) is the square of the correlation coefficient and measures how well the data 'fits' the regression line. For example, an r^2 value of 1 means that

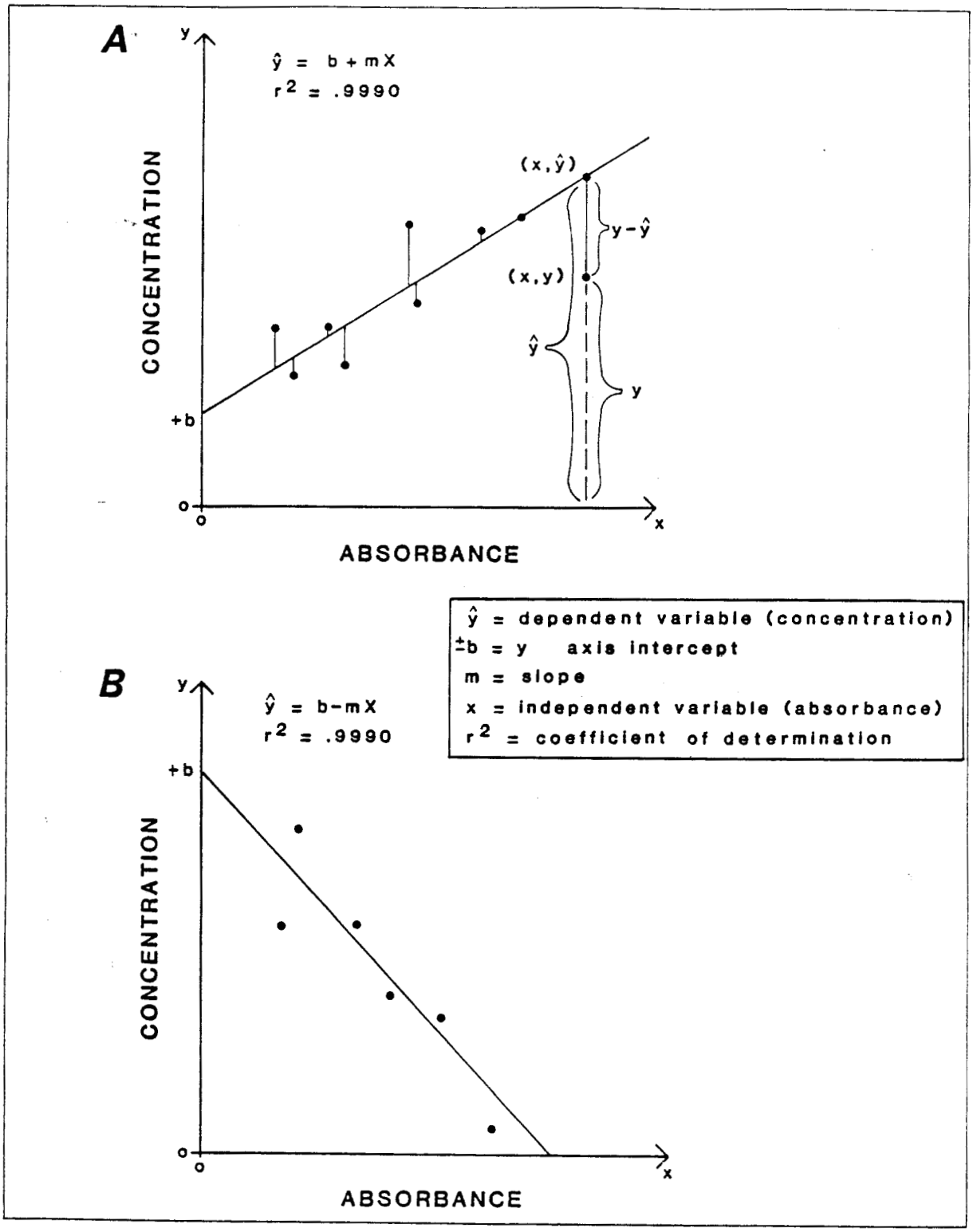


Figure 31. Description of linear regression showing (A) a positve correlation between concentration and absorbance, and (B) a negative correlation, and the general form of the regression equation.

all the data points lie on the regression line; i.e., 100% of the variation in Y is accounted for by its regression on X. An r^2 value of 0 means there is no correlation between the X and Y variables. For our analytical methods, r^2 values >.9990 are desired.

Finally, the relationship between the X and Y variables deviate from linearity above or below certain concentrations due to limitations in the analytical method; e.g., reagent strength, sample volume, and instrumentation. Therefore, it is crucial to use the linear portion of the calibration curve when calculating sample concentrations.

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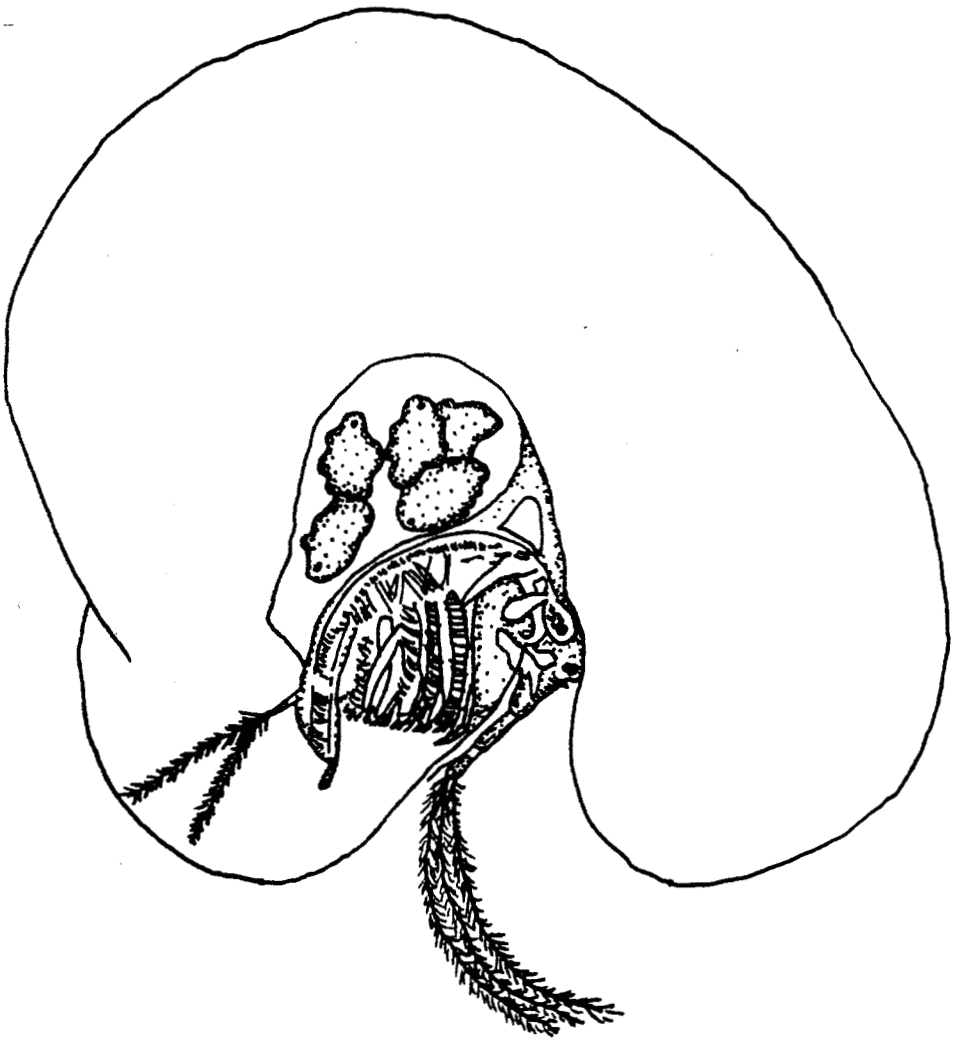
APPENDIX

DAILY TIMES FOR SUNRISE AND SUNSET AT VARIOUS GEOGRAPHIC LOCATIONS WITHIN ALASKA: USED TO CALCULATE THE DAY LENGTH FACTOR IN DETERMINING DAY RATE ESTIMATES OF PHOTOSYNTHESIS



TABLES OF SUNRISE AND SUNSET

SECOND PRINTING

The table on the other side of this sheet may be used in any year of the twentieth century and within the geographical boundary of the stated place with an error not exceeding 2 minutes and generally less than 1 minute. It may also be used anywhere in the vicinity of the stated place with an additional error of less than 1 minute for each nine miles, reckoned from the station of the U.S. Weather Bureau for the stated place, or reckoned from the nearest boundary in cases when no station of the Weather Bureau existed for the stated place.

Tables are available for almost all stations of the U.S. Weather Bureau, and all cities of over 50,000 population (1950 census) which are sufficiently remote from other cities of like size to require a separate computation.

The standard time shown is in conformity with time zone boundaries specified by the Interstate Commerce Commission as of 1 June 1969.

Eastern Standard Time is the local time of the 75th meridian. Central Standard Time is the local time of the 90th meridian. Mountain Standard Time is the local time of the 105th meridian. Pacific Standard Time is the local time of the 120th meridian.

Sunrise and sunset are considered to occur when the upper edge of the disk of the Sun appears to be exactly on the horizon. The times of sunrise and sunset given in this table are for an unobstructed horizon, with normal atmospheric conditions, at zero elevation above the Earth's surface in a level region.

The computations are based on a constant semidiameter of the Sun of 16 minutes of arc, an adopted refraction at the horizon of 34 minutes of arc, and the path of the Sun for the year 1966.

Should greater precision be required, corrections for elevation of the observer, angular elevation of the visible horizon, deviations from standard atmospheric conditions, and for a specific year may be derived from "Tables of Sunrise, Sunset and Twilight," Supplement to the American Ephemeris, 1946, obtainable from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402.

SUNRISE AND SUNSET AT ANCHORAGE, ALASKA

STANDARD TIME OF THE 150th MERIDIAN WEST.

NO.1005

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	9 14	2 54	8 21	4 07	7 01	5 25	5 24	6 45	3 52	8 04	2 37	9 20	2 28	9 39	3 32	8 39	4 51	7 07	6 06	5 32	7 27	3 59	8 45	2 53
2	9 13	2 55	8 18	4 10	6 58	5 27	5 21	6 48	3 49	8 07	2 35	9 22	2 29	9 38	3 34	8 36	4 54	7 04	6 08	5 29	7 30	3 56	8 47	2 51
3	9 12	2 57	8 15	4 13	6 55	5 30	5 17	6 51	3 46	8 09	2 34	9 23	2 30	9 37	3 37	8 33	4 56	7 01	6 11	5 26	7 33	3 53	8 49	2 50
4	9 12	2 59	8 13	4 16	6 52	5 33	5 14	6 53	3 43	8 12	2 32	9 25	2 32	9 36	3 40	8 31	4 59	6 57	6 13	5 22	7 35	3 51	8 51	2 49
5	9 11	3 01	8 10	4 19	6 49	5 35	5 11	6 56	3 40	8 15	2 31	9 27	2 33	9 35	3 42	8 28	5 01	6 54	6 16	5 19	7 38	3 48	8 53	2 48
6	9 10	3 02	8 08	4 21	6 46	5 38	5 08	6 58	3 38	8 17	2 30	9 29	2 35	9 33	3 45	8 25	5 04	6 51	6 19	5 16	7 41	3 45	8 55	2 47
7	9 09	3 04	8 05	4 24	6 43	5 41	5 05	7 01	3 35	8 20	2 28	9 30	2 36	9 32	3 47	8 22	5 06	6 48	6 21	5 13	7 44	3 43	8 57	2 46
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31	8 23	4 05			5 27	6 43			2 39	9 18			3 29	8 41	4 49	7 10			7 24	4 02			9 14	2 52

Add one hour for Daylight Saving Time if and when in use.

Prepared by
NAUTICAL ALMANAC OFFICE
UNITED STATES NAVAL OBSERVATORY
WASHINGTON, D.C. 20390

SUNRISE AND SUNSET AT ANNETTE ISLAND, ALASKA

NO. 1006

PACIFIC STANDARD TIME

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
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17	9 00	4 53	8 05	5 57	6 57	6 54	5 39	7 54	4 35	8 51	4 06	9 28	4 30	9 14	5 24	8 15	6 22	6 58	7 19	5 43	8 22	4 40	9 07	4 18
18	8 59	4 55	8 03	5 59	6 54	6 56	5 36	7 56	4 34	8 53	4 06	9 29	4 31	9 13	5 26	8 13	6 24	6 56	7 21	5 41	8 24	4 38	9 08	4 18
19	8 58	4 57	8 00	6 01	6 52	6 58	5 34	7 58	4 32	8 55	4 06	9 29	4 33	9 11	5 28	8 11	6 26	6 53	7 23	5 38	8 26	4 37	9 08	4 18
20	8 56	4 59	7 58	6 03	6 49	7 00	5 31	8 00	4 30	8 56	4 06	9 29	4 34	9 10	5 29	8 08	6 28	6 51	7 25	5 36	8 28	4 35	9 09	4 19
21	8 55	5 01	7 56	6 05	6 47	7 02	5 29	8 02	4 29	8 58	4 06	9 29	4 36	9 08	5 31	8 06	6 29	6 48	7 27	5 33	8 30	4 34	9 10	4 19
22	8 54	5 03	7 53	6 07	6 44	7 04	5 27	8 04	4 27	8 59	4 07	9 30	4 37	9 07	5 33	8 04	6 31	6 46	7 29	5 31	8 32	4 33	9 10	4 20
23	8 52	5 05	7 51	6 09	6 41	7 06	5 24	8 06	4 26	9 01	4 07	9 30	4 39	9 05	5 35	8 01	6 33	6 43	7 31	5 29	8 34	4 31	9 11	4 20
24	8 51	5 07	7 49	6 11	6 39	7 07	5 22	8 08	4 24	9 03	4 07	9 30	4 41	9 04	5 37	7 59	6 35	6 40	7 34	5 27	8 35	4 30	9 11	4 21
25	8 49	5 09	7 46	6 13	6 36	7 09	5 20	8 10	4 23	9 04	4 08	9 30	4 42	9 02	5 39	7 56	6 37	6 38	7 36	5 24	8 37	4 29	9 11	4 22
26	8 48	5 11	7 44	6 16	6 34	7 11	5 18	8 12	4 22	9 06	4 08	9 30	4 44	9 00	5 41	7 54	6 39	6 35	7 38	5 22	8 39	4 28	9 12	4 22
27	8 46	5 13	7 42	6 18	6 31	7 13	5 15	8 14	4 20	9 07	4 09	9 30	4 46	8 59	5 43	7 52	6 41	6 33	7 40	5 20	8 41	4 27	9 12	4 23
28	8 44	5 15	7 39	6 20	6 29	7 15	5 13	8 16	4 19	9 09	4 09	9 29	4 47	8 57	5 45	7 49	6 43	6 30	7 42	5 18	8 42	4 26	9 12	4 24
29	8 43	5 17	7 38	6 21	6 26	7 17	5 11	8 18	4 18	9 10	4 10	9 29	4 49	8 55	5 46	7 47	6 44	6 28	7 44	5 16	8 44	4 25	9 12	4 25
30	8 41	5 19			6 24	7 19	5 09	8 20	4 17	9 11	4 11	9 29	4 51	8 53	5 48	7 44	6 46	6 25	7 46	5 13	8 46	4 24	9 12	4 26
31	8 39	5 21			6 21	7 21			4 16	9 13			4 53	8 51	5 50	7 42			7 48	5 11			9 12	4 27

Add one hour for Daylight Saving Time if and when in use.

I certify that the above data are the result of an accurate and true computation by the Nautical Almanac Office, United States Naval Observatory, an agency charged by Federal Statute (9 Stat. L 374, 375) with the duty of making such computations and publishing the results.

E. W. WOOLARD
Director Nautical Almanac
U. S. Naval Observatory

C. G. CHRISTIE
Captain, USN

SUNRISE AND SUNSET AT CORDOVA, ALASKA

STANDARD TIME OF THE 150th MERIDIAN WEST

NO. 1011

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	8 49	2 42	7 59	3 53	6 42	5 08	5 07	6 26	3 38	7 42	2 26	8 54	2 18	9 13	3 19	8 16	4 35	6 47	5 47	5 15	7 06	3 44	8 21	2 41
2	8 49	2 44	7 56	3 56	6 39	5 11	5 04	6 29	3 35	7 45	2 25	8 56	2 19	9 12	3 21	8 13	4 38	6 44	5 50	5 11	7 09	3 41	8 23	2 39
3	8 48	2 46	7 54	3 59	6 36	5 13	5 01	6 31	3 32	7 47	2 23	8 58	2 20	9 11	3 24	8 11	4 40	6 41	5 52	5 08	7 11	3 39	8 25	2 38
4	8 47	2 47	7 51	4 01	6 33	5 16	4 58	6 34	3 29	7 50	2 22	9 00	2 22	9 10	3 26	8 08	4 42	6 38	5 55	5 05	7 14	3 36	8 27	2 37
5	8 46	2 49	7 49	4 04	6 30	5 19	4 55	6 36	3 27	7 52	2 21	9 01	2 23	9 09	3 29	8 06	4 45	6 35	5 57	5 02	7 17	3 34	8 29	2 36
6	8 45	2 51	7 46	4 07	6 27	5 21	4 52	6 39	3 24	7 55	2 19	9 03	2 25	9 08	3 31	8 03	4 47	6 32	6 00	4 59	7 19	3 31	8 31	2 35
7	8 44	2 53	7 44	4 10	6 24	5 24	4 48	6 41	3 21	7 58	2 18	9 04	2 26	9 06	3 33	8 00	4 50	6 29	6 02	4 56	7 22	3 29	8 33	2 34
8	8 43	2 55	7 41	4 12	6 21	5 26	4 45	6 44	3 19	8 00	2 17	9 06	2 28	9 05	3 36	7 57	4 52	6 26	6 05	4 53	7 25	3 26	8 34	2 33
9	8 42	2 57	7 39	4 15	6 18	5 29	4 42	6 46	3 16	8 03	2 16	9 07	2 29	9 04	3 38	7 55	4 54	6 23	6 07	4 50	7 27	3 24	8 36	2 33
10	8 41	2 59	7 36	4 18	6 15	5 31	4 39	6 49	3 13	8 05	2 15	9 08	2 31	9 02	3 41	7 52	4 57	6 19	6 10	4 47	7 30	3 21	8 38	2 32
11	8 40	3 01	7 33	4 20	6 12	5 34	4 36	6 51	3 11	8 08	2 15	9 09	2 33	9 01	3 43	7 49	4 59	6 16	6 12	4 44	7 33	3 19	8 39	2 31
12	8 38	3 03	7 30	4 23	6 09	5 36	4 33	6 54	3 08	8 10	2 14	9 10	2 35	8 59	3 46	7 46	5 02	6 13	6 15	4 41	7 35	3 16	8 40	2 31
13	8 37	3 05	7 28	4 26	6 06	5 39	4 30	6 56	3 06	8 13	2 13	9 11	2 36	8 57	3 48	7 43	5 04	6 10	6 17	4 38	7 38	3 14	8 42	2 31
14	8 35	3 08	7 25	4 29	6 02	5 41	4 27	6 59	3 03	8 15	2 13	9 12	2 38	8 56	3 51	7 41	5 06	6 07	6 20	4 35	7 40	3 12	8 43	2 30
15	8 34	3 10	7 22	4 31	5 59	5 44	4 24	7 02	3 01	8 17	2 12	9 13	2 40	8 54	3 53	7 38	5 09	6 04	6 22	4 32	7 43	3 10	8 44	2 30
16	8 32	3 12	7 19	4 34	5 56	5 46	4 21	7 04	2 58	8 20	2 12	9 14	2 42	8 52	3 56	7 35	5 11	6 01	6 25	4 29	7 46	3 07	8 45	2 30
17	8 30	3 15	7 17	4 37	5 53	5 49	4 18	7 07	2 56	8 22	2 12	9 14	2 44	8 50	3 58	7 32	5 14	5 58	6 27	4 26	7 48	3 05	8 46	2 30
18	8 29	3 17	7 14	4 39	5 50	5 51	4 15	7 09	2 54	8 25	2 11	9 15	2 46	8 48	4 01	7 29	5 16	5 55	6 30	4 23	7 51	3 03	8 47	2 30
19	8 27	3 19	7 11	4 42	5 47	5 54	4 12	7 12	2 51	8 27	2 11	9 15	2 49	8 46	4 03	7 26	5 18	5 52	6 32	4 20	7 53	3 01	8 48	2 31
20	8 25	3 22	7 08	4 45	5 44	5 56	4 09	7 14	2 49	8 29	2 11	9 16	2 51	8 44	4 06	7 23	5 21	5 48	6 35	4 18	7 56	2 59	8 48	2 31
21	8 23	3 24	7 05	4 47	5 41	5 59	4 06	7 17	2 47	8 32	2 12	9 16	2 53	8 42	4 08	7 20	5 23	5 45	6 37	4 15	7 58	2 57	8 49	2 31
22	8 21	3 27	7 02	4 50	5 38	6 01	4 03	7 19	2 45	8 34	2 12	9 16	2 55	8 40	4 11	7 17	5 26	5 42	6 40	4 12	8 01	2 55	8 50	2 32
23	8 19	3 30	6 59	4 53	5 35	6 04	4 00	7 22	2 43	8 36	2 12	9 16	2 57	8 38	4 13	7 14	5 28	5 39	6 43	4 09	8 03	2 53	8 50	2 32
24	8 17	3 32	6 57	4 55	5 32	6 06	3 58	7 24	2 41	8 38	2 13	9 16	3 00	8 35	4 16	7 11	5 31	5 36	6 45	4 06	8 05	2 51	8 50	2 33
25	8 15	3 35	6 54	4 58	5 29	6 09	3 55	7 27	2 39	8 41	2 13	9 16	3 02	8 33	4 18	7 08	5 33	5 33	6 48	4 03	8 08	2 50	8 51	2 34
26	8 13	3 37	6 51	5 00	5 25	6 11	3 52	7 30	2 37	8 43	2 14	9 15	3 04	8 31	4 20	7 05	5 35	5 30	6 50	4 01	8 10	2 48	8 51	2 35
27	8 10	3 40	6 48	5 03	5 22	6 14	3 49	7 32	2 35	8 45	2 14	9 15	3 07	8 28	4 23	7 02	5 38	5 27	6 53	3 58	8 12	2 46	8 51	2 36
28	8 08	3 43	6 45	5 06	5 19	6 16	3 46	7 35	2 33	8 47	2 15	9 15	3 09	8 26	4 25	6 59	5 40	5 24	6 56	3 55	8 15	2 45	8 51	2 37
29	8 06	3 45	6 44	5 07	5 16	6 19	3 43	7 37	2 31	8 49	2 16	9 14	3 11	8 24	4 28	6 56	5 43	5 21	6 58	3 52	8 17	2 43	8 50	2 38
30	8 04	3 48			5 13	6 21	3 40	7 40	2 30	8 51	2 17	9 13	3 14	8 21	4 30	6 53	5 45	5 18	7 01	3 49	8 19	2 42	8 50	2 39
31	8 01	3 51			5 10	6 24			2 28	8 53			3 16	8 19	4 33	6 50			7 04	3 47			8 50	2 41

Add one hour for Daylight Saving Time if and when in use.

I certify that the above data are the result of an accurate and true computation by the Nautical Almanac Office, United States Naval Observatory, an agency charged by Federal Statute (9 Stat. L 374, 375) with the duty of making such computations and publishing the results.

E. W. WOOLARD
Director Nautical Almanac
U. S. Naval Observatory

C. G. CHRISTIE

SUNRISE AND SUNSET AT FAIRBANKS, ALASKA

STANDARD TIME OF THE 150th MERIDIAN WEST.

NO. 1012

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	9 55	1 56	8 38	3 34	7 01	5 08	5 09	6 44	3 19	8 20	1 34	10 07	1 11	10 37	2 50	9 02	4 31	7 10	6 00	5 20	7 39	3 31	9 19	2 02
2	9 53	1 58	8 34	3 37	6 58	5 11	5 05	6 47	3 15	8 24	1 31	10 10	1 13	10 35	2 54	8 59	4 34	7 06	6 03	5 17	7 42	3 27	9 22	2 00
3	9 52	2 00	8 31	3 41	6 54	5 14	5 01	6 50	3 12	8 27	1 28	10 13	1 16	10 33	2 57	8 55	4 37	7 03	6 07	5 13	7 45	3 24	9 25	1 57
4	9 51	2 03	8 28	3 44	6 51	5 17	4 58	6 53	3 08	8 31	1 25	10 17	1 19	10 31	3 01	8 52	4 40	6 59	6 10	5 09	7 49	3 20	9 28	1 55
5	9 49	2 05	8 25	3 48	6 47	5 20	4 54	6 56	3 05	8 34	1 23	10 20	1 21	10 28	3 04	8 48	4 43	6 55	6 13	5 06	7 52	3 17	9 30	1 54
6	9 47	2 08	8 21	3 51	6 43	5 24	4 50	6 59	3 01	8 37	1 20	10 22	1 24	10 26	3 07	8 45	4 46	6 52	6 16	5 02	7 55	3 14	9 33	1 52
7	9 45	2 11	8 18	3 55	6 40	5 27	4 47	7 03	2 58	8 41	1 18	10 25	1 27	10 23	3 11	8 41	4 49	6 48	6 19	4 58	7 59	3 11	9 36	1 50
8	9 43	2 14	8 15	3 58	6 36	5 30	4 43	7 06	2 54	8 44	1 15	10 28	1 30	10 21	3 14	8 37	4 52	6 44	6 22	4 55	8 02	3 07	9 38	1 48
9	9 41	2 17	8 11	4 01	6 33	5 33	4 39	7 09	2 50	8 48	1 13	10 30	1 33	10 18	3 17	8 34	4 55	6 41	6 25	4 51	8 06	3 04	9 40	1 47
10	9 39	2 19	8 08	4 05	6 29	5 36	4 36	7 12	2 47	8 51	1 11	10 33	1 36	10 15	3 21	8 30	4 58	6 37	6 28	4 48	8 09	3 01	9 43	1 46
11	9 37	2 23	8 04	4 08	6 25	5 39	4 32	7 15	2 43	8 55	1 09	10 35	1 39	10 12	3 24	8 27	5 01	6 33	6 31	4 44	8 12	2 58	9 45	1 44
12	9 35	2 26	8 01	4 12	6 22	5 42	4 28	7 18	2 40	8 58	1 07	10 37	1 42	10 09	3 27	8 23	5 04	6 30	6 34	4 40	8 16	2 54	9 47	1 43
13	9 32	2 29	7 58	4 15	6 18	5 45	4 25	7 21	2 36	9 02	1 05	10 39	1 46	10 06	3 31	8 19	5 07	6 26	6 37	4 37	8 19	2 51	9 49	1 42
14	9 30	2 32	7 54	4 19	6 14	5 49	4 21	7 25	2 33	9 05	1 03	10 41	1 49	10 03	3 34	8 16	5 10	6 22	6 40	4 33	8 23	2 48	9 51	1 42
15	9 27	2 35	7 51	4 22	6 11	5 52	4 17	7 28	2 29	9 09	1 02	10 43	1 52	10 00	3 37	8 12	5 13	6 19	6 43	4 30	8 26	2 45	9 52	1 41
16	9 25	2 38	7 47	4 25	6 07	5 55	4 14	7 31	2 26	9 12	1 01	10 44	1 56	9 57	3 40	8 09	5 16	6 15	6 47	4 26	8 30	2 42	9 54	1 41
17	9 22	2 42	7 44	4 29	6 04	5 58	4 10	7 34	2 22	9 16	1 00	10 45	1 59	9 53	3 44	8 05	5 19	6 11	6 50	4 23	8 33	2 39	9 55	1 40
18	9 20	2 45	7 40	4 32	6 00	6 01	4 06	7 37	2 19	9 19	12 59	10 46	2 02	9 50	3 47	8 01	5 22	6 08	6 53	4 19	8 37	2 36	9 56	1 40
19	9 17	2 49	7 37	4 35	5 56	6 04	4 03	7 41	2 16	9 23	12 59	10 47	2 06	9 47	3 50	7 58	5 25	6 04	6 56	4 16	8 40	2 33	9 57	1 40
20	9 14	2 52	7 33	4 39	5 53	6 07	3 59	7 44	2 12	9 26	12 59	10 48	2 09	9 44	3 53	7 54	5 28	6 00	6 59	4 12	8 43	2 30	9 58	1 40
21	9 11	2 55	7 30	4 42	5 49	6 10	3 55	7 47	2 09	9 30	12 59	10 48	2 13	9 40	3 56	7 50	5 31	5 57	7 02	4 09	8 47	2 27	9 59	1 41
22	9 08	2 59	7 26	4 45	5 45	6 13	3 52	7 50	2 06	9 33	12 59	10 48	2 16	9 37	4 00	7 47	5 34	5 53	7 06	4 05	8 50	2 25	9 59	1 41
23	9 05	3 02	7 23	4 48	5 42	6 16	3 48	7 54	2 02	9 37	12 59	10 47	2 19	9 34	4 03	7 43	5 37	5 49	7 09	4 02	8 53	2 22	9 59	1 42
24	9 02	3 06	7 19	4 52	5 38	6 19	3 44	7 57	1 59	9 40	1 00	10 47	2 23	9 30	4 06	7 39	5 40	5 46	7 12	3 58	8 57	2 19	10 00	1 43
25	8 59	3 09	7 16	4 55	5 34	6 22	3 41	8 00	1 56	9 44	1 01	10 46	2 26	9 27	4 09	7 36	5 43	5 42	7 15	3 55	9 00	2 16	10 00	1 44
26	8 56	3 13	7 12	4 58	5 31	6 25	3 37	8 04	1 52	9 47	1 02	10 45	2 30	9 23	4 12	7 32	5 46	5 38	7 19	3 51	9 03	2 14	9 59	1 45
27	8 53	3 16	7 08	5 01	5 27	6 29	3 34	8 07	1 49	9 51	1 04	10 44	2 33	9 20	4 15	7 28	5 49	5 35	7 22	3 48	9 06	2 11	9 59	1 46
28	8 50	3 20	7 05	5 05	5 23	6 32	3 30	8 10	1 46	9 54	1 05	10 43	2 37	9 16	4 18	7 25	5 51	5 31	7 25	3 44	9 10	2 09	9 59	1 48
29	8 47	3 23	7 03	5 07	5 20	6 35	3 26	8 14	1 43	9 57	1 07	10 41	2 40	9 13	4 22	7 21	5 54	5 27	7 29	3 41	9 13	2 06	9 58	1 50
30	8 44	3 27			5 16	6 38	3 23	8 17	1 40	10 01	1 09	10 39	2 44	9 09	4 25	7 17	5 57	5 24	7 32	3 37	9 16	2 04	9 57	1 51
31	8 41	3 30			5 12	6 41			1 37	10 04			2 47	9 06	4 28	7 14			7 35	3 34			9 56	1 53

Add one hour for Daylight Saving Time if and when in use.

Prepared by
NAUTICAL ALMANAC OFFICE
UNITED STATES NAVAL OBSERVATORY

SUNRISE AND SUNSET AT JUNEAU, ALASKA

PACIFIC STANDARD TIME

NO. 1013

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	9 47	4 17	9 04	5 21	7 54	6 28	6 26	7 39	5 05	8 48	4 02	9 51	3 57	10 07	4 49	9 19	5 57	7 58	7 02	6 32	8 14	5 09	9 21	4 14
2	9 46	4 19	9 02	5 23	7 51	6 31	6 24	7 42	5 02	8 50	4 01	9 52	3 58	10 06	4 51	9 17	5 59	7 55	7 05	6 30	8 16	5 07	9 22	4 13
3	9 46	4 20	9 00	5 26	7 49	6 33	6 21	7 44	5 00	8 52	4 00	9 54	3 59	10 06	4 53	9 14	6 01	7 53	7 07	6 27	8 18	5 05	9 24	4 12
4	9 45	4 22	8 58	5 28	7 46	6 35	6 18	7 46	4 57	8 55	3 59	9 55	4 00	10 05	4 55	9 12	6 03	7 50	7 09	6 24	8 21	5 02	9 26	4 11
5	9 45	4 23	8 55	5 30	7 43	6 38	6 15	7 49	4 55	8 57	3 58	9 56	4 01	10 04	4 57	9 10	6 06	7 47	7 11	6 21	8 23	5 00	9 28	4 10
6	9 44	4 25	8 53	5 33	7 40	6 40	6 12	7 51	4 52	8 59	3 57	9 58	4 02	10 03	5 00	9 07	6 08	7 44	7 13	6 18	8 25	4 58	9 29	4 09
7	9 43	4 27	8 51	5 35	7 38	6 42	6 09	7 53	4 50	9 01	3 56	9 59	4 03	10 02	5 02	9 05	6 10	7 41	7 16	6 16	8 28	4 55	9 31	4 09
8	9 42	4 28	8 48	5 38	7 35	6 45	6 07	7 55	4 48	9 03	3 55	10 00	4 05	10 01	5 04	9 02	6 12	7 38	7 18	6 13	8 30	4 53	9 32	4 08
9	9 41	4 30	8 46	5 40	7 32	6 47	6 04	7 58	4 45	9 06	3 54	10 01	4 06	10 00	5 06	9 00	6 14	7 36	7 20	6 10	8 33	4 51	9 34	4 07
10	9 40	4 32	8 44	5 43	7 29	6 49	6 01	8 00	4 43	9 08	3 54	10 02	4 08	9 59	5 08	8 57	6 16	7 33	7 22	6 07	8 35	4 49	9 35	4 07
11	9 39	4 34	8 41	5 45	7 26	6 52	5 58	8 02	4 41	9 10	3 53	10 03	4 09	9 57	5 11	8 55	6 19	7 30	7 25	6 04	8 37	4 47	9 37	4 07
12	9 38	4 36	8 39	5 48	7 23	6 54	5 55	8 04	4 38	9 12	3 52	10 04	4 11	9 56	5 13	8 52	6 21	7 27	7 27	6 02	8 40	4 45	9 38	4 06
13	9 37	4 38	8 36	5 50	7 21	6 56	5 53	8 07	4 36	9 14	3 52	10 05	4 12	9 55	5 15	8 50	6 23	7 24	7 29	6 59	8 42	4 43	9 39	4 06
14	9 36	4 40	8 34	5 52	7 18	6 59	5 50	8 09	4 34	9 17	3 52	10 06	4 14	9 53	5 17	8 47	6 25	7 21	7 32	6 56	8 44	4 41	9 40	4 06
15	9 34	4 42	8 31	5 55	7 15	7 01	5 47	8 11	4 32	9 19	3 51	10 06	4 16	9 52	5 19	8 45	6 27	7 18	7 34	6 53	8 47	4 39	9 41	4 06
16	9 33	4 44	8 29	5 57	7 12	7 03	5 44	8 13	4 30	9 21	3 51	10 07	4 17	9 50	5 22	8 42	6 29	7 15	7 36	5 51	8 49	4 37	9 42	4 06
17	9 32	4 46	8 26	6 00	7 09	7 05	5 42	8 16	4 28	9 23	3 51	10 08	4 19	9 48	5 24	8 39	6 32	7 13	7 38	5 48	8 51	4 35	9 43	4 06
18	9 30	4 48	8 24	6 02	7 06	7 08	5 39	8 18	4 26	9 25	3 51	10 08	4 21	9 47	5 26	8 37	6 34	7 10	7 41	5 45	8 53	4 33	9 44	4 06
19	9 29	4 50	8 21	6 05	7 04	7 10	5 36	8 20	4 24	9 27	3 51	10 08	4 23	9 45	5 28	8 34	6 36	7 07	7 43	5 43	8 56	4 31	9 45	4 06
20	9 27	4 53	8 18	6 07	7 01	7 12	5 33	8 23	4 22	9 29	3 51	10 09	4 25	9 43	5 30	8 31	6 38	7 04	7 45	5 40	8 58	4 29	9 45	4 07
21	9 25	4 55	8 16	6 09	6 58	7 15	5 31	8 25	4 20	9 31	3 51	10 09	4 26	9 42	5 33	8 29	6 40	7 01	7 48	5 37	9 00	4 28	9 46	4 07
22	9 24	4 57	8 13	6 12	6 55	7 17	5 28	8 27	4 18	9 33	3 51	10 09	4 28	9 40	5 35	8 26	6 43	6 58	7 50	5 35	9 02	4 26	9 46	4 08
23	9 22	4 59	8 10	6 14	6 52	7 19	5 25	8 29	4 16	9 35	3 51	10 09	4 30	9 38	5 37	8 23	6 45	6 55	7 52	5 32	9 04	4 25	9 47	4 08
24	9 20	5 02	8 08	6 17	6 49	7 21	5 23	8 32	4 15	9 37	3 52	10 09	4 32	9 36	5 39	8 21	6 47	6 52	7 55	5 29	9 07	4 23	9 47	4 09
25	9 18	5 04	8 05	6 19	6 46	7 24	5 20	8 34	4 13	9 39	3 52	10 09	4 34	9 34	5 41	8 18	6 49	6 50	7 57	5 27	9 09	4 21	9 47	4 10
26	9 16	5 06	8 02	6 21	6 44	7 26	5 17	8 36	4 11	9 41	3 53	10 09	4 36	9 32	5 44	8 15	6 51	6 47	7 59	5 24	9 11	4 20	9 48	4 11
27	9 14	5 09	8 00	6 24	6 41	7 28	5 15	8 39	4 10	9 42	3 53	10 09	4 38	9 30	5 46	8 12	6 53	6 44	8 02	5 22	9 13	4 19	9 48	4 11
28	9 12	5 11	7 57	6 26	6 38	7 30	5 12	8 41	4 08	9 44	3 54	10 08	4 40	9 28	5 48	8 09	6 56	6 41	8 04	5 19	9 15	4 17	9 48	4 12
29	9 10	5 13	7 56	6 27	6 35	7 33	5 10	8 43	4 07	9 46	3 55	10 08	4 42	9 25	5 50	8 07	6 58	6 38	8 06	5 17	9 17	4 16	9 48	4 13
30	9 08	5 16			6 32	7 35	5 07	8 45	4 05	9 47	3 56	10 07	4 45	9 23	5 52	8 04	7 00	6 35	8 09	5 14	9 19	4 15	9 47	4 15
31	9 06	5 18			6 29	7 37			4 04	9 49			4 47	9 21	5 55	8 01			8 11	5 12			9 47	4 16

Add one hour for Daylight Saving Time if and when in use.

I certify that the above data are the result of an accurate and true computation by the Nautical Almanac Office, United States Naval Observatory, an agency charged by Federal Statute (9 Stat. L 374, 375) with the duty of making such computations and publishing the results.

E W Woolard

E. W. WOOLARD
Director Nautical Almanac
U. S. Naval Observatory

C. R. Christie

SUNRISE AND SUNSET AT KING SALMON, ALASKA

NO. 1014

STANDARD TIME OF THE 150th MERIDIAN WEST.

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	9 18	3 43	8 34	4 48	7 23	5 56	5 54	7 08	4 31	8 18	3 28	9 22	3 22	9 38	4 15	8 49	5 24	7 27	6 31	6 00	7 43	4 36	8 51	3 40
2	9 17	3 45	8 32	4 50	7 20	5 59	5 51	7 11	4 29	8 20	3 27	9 23	3 23	9 37	4 17	8 47	5 27	7 24	6 33	5 57	7 46	4 34	8 53	3 39
3	9 17	3 46	8 29	4 53	7 17	6 01	5 48	7 13	4 26	8 22	3 25	9 25	3 24	9 37	4 20	8 44	5 29	7 21	6 35	5 55	7 48	4 31	8 55	3 38
4	9 16	3 48	8 27	4 55	7 14	6 03	5 45	7 15	4 24	8 25	3 24	9 26	3 25	9 36	4 22	8 42	5 31	7 19	6 38	5 52	7 50	4 29	8 57	3 37
5	9 15	3 49	8 25	4 57	7 12	6 06	5 43	7 17	4 21	8 27	3 23	9 28	3 26	9 35	4 24	8 39	5 33	7 16	6 40	5 49	7 53	4 27	8 58	3 36
6	9 15	3 51	8 22	5 00	7 09	6 08	5 40	7 20	4 19	8 29	3 22	9 29	3 28	9 34	4 26	8 37	5 36	7 13	6 42	5 46	7 55	4 24	9 00	3 35
7	9 14	3 53	8 20	5 02	7 06	6 11	5 37	7 22	4 16	8 31	3 21	9 30	3 29	9 33	4 28	8 34	5 38	7 10	6 44	5 43	7 58	4 22	9 02	3 34
8	9 13	3 54	8 18	5 05	7 03	6 13	5 34	7 24	4 14	8 34	3 20	9 31	3 30	9 32	4 31	8 32	5 40	7 07	6 47	5 40	8 00	4 20	9 03	3 34
9	9 12	3 56	8 15	5 07	7 00	6 15	5 31	7 27	4 12	8 36	3 20	9 33	3 32	9 31	4 33	8 30	5 42	7 04	6 49	5 38	8 03	4 18	9 05	3 33
10	9 11	3 58	8 13	5 10	6 57	6 18	5 28	7 29	4 09	8 38	3 19	9 34	3 33	9 29	4 35	8 27	5 44	7 01	6 51	5 35	8 05	4 15	9 06	3 33
11	9 10	4 00	8 10	5 12	6 55	6 20	5 25	7 31	4 07	8 40	3 18	9 35	3 35	9 28	4 37	8 24	5 47	6 58	6 54	5 32	8 07	4 13	9 07	3 32
12	9 09	4 02	8 08	5 15	6 52	6 22	5 23	7 34	4 05	8 43	3 18	9 36	3 36	9 27	4 40	8 22	5 49	6 55	6 56	5 29	8 10	4 11	9 09	3 32
13	9 07	4 04	8 05	5 17	6 49	6 25	5 20	7 36	4 02	8 45	3 17	9 36	3 38	9 25	4 42	8 19	5 51	6 53	6 58	5 26	8 12	4 09	9 10	3 32
14	9 06	4 06	8 03	5 20	6 46	6 27	5 17	7 38	4 00	8 47	3 17	9 37	3 40	9 24	4 44	8 17	5 53	6 50	7 01	5 24	8 14	4 07	9 11	3 32
15	9 05	4 08	8 00	5 22	6 43	6 29	5 14	7 41	3 58	8 49	3 16	9 38	3 41	9 22	4 46	8 14	5 55	6 47	7 03	5 21	8 17	4 05	9 12	3 31
16	9 03	4 10	7 58	5 25	6 40	6 32	5 11	7 43	3 56	8 51	3 16	9 38	3 43	9 21	4 49	8 11	5 58	6 44	7 05	5 18	8 19	4 03	9 13	3 31
17	9 02	4 12	7 55	5 27	6 37	6 34	5 09	7 45	3 54	8 53	3 16	9 39	3 45	9 19	4 51	8 09	6 00	6 41	7 08	5 15	8 21	4 01	9 14	3 32
18	9 00	4 15	7 53	5 30	6 35	6 36	5 06	7 47	3 52	8 56	3 16	9 39	3 47	9 17	4 53	8 06	6 02	6 38	7 10	5 13	8 24	3 59	9 15	3 32
19	8 59	4 17	7 50	5 32	6 32	6 38	5 03	7 50	3 50	8 58	3 16	9 40	3 49	9 16	4 55	8 03	6 04	6 35	7 12	5 10	8 26	3 57	9 16	3 32
20	8 57	4 19	7 47	5 35	6 29	6 41	5 00	7 52	3 48	9 00	3 16	9 40	3 51	9 14	4 58	8 01	6 06	6 32	7 15	5 07	8 28	3 56	9 16	3 32
21	8 55	4 21	7 45	5 37	6 26	6 43	4 58	7 54	3 46	9 02	3 16	9 40	3 52	9 12	5 00	7 58	6 09	6 29	7 17	5 05	8 31	3 54	9 17	3 33
22	8 54	4 24	7 42	5 39	6 23	6 45	4 55	7 57	3 44	9 04	3 16	9 41	3 54	9 10	5 02	7 55	6 11	6 26	7 19	5 02	8 33	3 52	9 17	3 33
23	8 52	4 26	7 39	5 42	6 20	6 48	4 52	7 59	3 42	9 06	3 17	9 41	3 56	9 08	5 04	7 52	6 13	6 23	7 22	4 59	8 35	3 51	9 18	3 34
24	8 50	4 28	7 37	5 44	6 17	6 50	4 50	8 01	3 40	9 08	3 17	9 41	3 58	9 06	5 07	7 50	6 15	6 21	7 24	4 57	8 37	3 49	9 18	3 35
25	8 48	4 31	7 34	5 47	6 14	6 52	4 47	8 04	3 39	9 10	3 18	9 40	4 00	9 04	5 09	7 47	6 18	6 18	7 26	4 54	8 39	3 48	9 18	3 35
26	8 46	4 33	7 31	5 49	6 11	6 55	4 44	8 06	3 37	9 11	3 18	9 40	4 03	9 02	5 11	7 44	6 20	6 15	7 29	4 51	8 41	3 46	9 18	3 36
27	8 44	4 35	7 28	5 51	6 09	6 57	4 42	8 08	3 35	9 13	3 19	9 40	4 05	9 00	5 13	7 41	6 22	6 12	7 31	4 49	8 43	3 45	9 19	3 37
28	8 42	4 38	7 26	5 54	6 06	6 59	4 39	8 11	3 34	9 15	3 19	9 40	4 07	8 58	5 16	7 38	6 24	6 09	7 34	4 46	8 45	3 43	9 19	3 38
29	8 40	4 40	7 25	5 55	6 03	7 01	4 36	8 13	3 32	9 17	3 20	9 39	4 09	8 56	5 18	7 36	6 26	6 06	7 36	4 44	8 47	3 42	9 18	3 39
30	8 38	4 43			6 00	7 04	4 34	8 15	3 31	9 19	3 21	9 39	4 11	8 53	5 20	7 33	6 29	6 03	7 38	4 41	8 49	3 41	9 18	3 40
31	8 36	4 45			5 57	7 06			3 29	9 20			4 13	8 51	5 22	7 30			7 41	4 39			9 18	3 42

Add one hour for Daylight Saving Time if and when in use.

Prepared by
NAUTICAL ALMANAC OFFICE
UNITED STATES NAVAL OBSERVATORY
WASHINGTON, D.C. 20390

SUNRISE AND SUNSET AT YAKUTAT, ALASKA

STANDARD TIME OF THE 135th MERIDIAN WEST

NO. 1019

DAY	JAN.		FEB.		MAR.		APR.		MAY		JUNE		JULY		AUG.		SEPT.		OCT.		NOV.		DEC.	
	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.	Rise A.M.	Set P.M.
1	9 17	3 28	8 30	4 35	7 17	5 47	5 45	7 01	4 19	8 14	3 13	9 21	3 06	9 38	4 02	8 46	5 14	7 21	6 23	5 52	7 39	4 25	8 50	3 25
2	9 16	3 30	8 28	4 38	7 14	5 49	5 42	7 04	4 17	8 16	3 11	9 23	3 07	9 38	4 04	8 44	5 17	7 18	6 26	5 49	7 41	4 23	8 52	3 24
3	9 16	3 31	8 26	4 40	7 11	5 52	5 39	7 06	4 14	8 19	3 10	9 25	3 08	9 37	4 07	8 41	5 19	7 15	6 28	5 46	7 44	4 20	8 53	3 23
4	9 15	3 33	8 23	4 43	7 08	5 54	5 36	7 09	4 12	8 21	3 09	9 26	3 09	9 36	4 09	8 39	5 21	7 13	6 30	5 43	7 46	4 18	8 55	3 22
5	9 14	3 34	8 21	4 46	7 05	5 57	5 33	7 11	4 09	8 23	3 08	9 28	3 10	9 35	4 11	8 36	5 24	7 10	6 33	5 40	7 49	4 15	8 57	3 21
6	9 13	3 36	8 18	4 48	7 02	5 59	5 30	7 13	4 06	8 26	3 06	9 29	3 12	9 34	4 14	8 34	5 26	7 07	6 35	5 37	7 51	4 13	8 59	3 20
7	9 13	3 38	8 16	4 51	6 59	6 01	5 27	7 16	4 04	8 28	3 05	9 30	3 13	9 33	4 16	8 31	5 28	7 04	6 37	5 34	7 54	4 10	9 01	3 20
8	9 12	3 40	8 13	4 53	6 56	6 04	5 24	7 18	4 01	8 31	3 04	9 32	3 14	9 32	4 18	8 29	5 30	7 01	6 40	5 31	7 56	4 08	9 02	3 19
9	9 11	3 42	8 11	4 56	6 53	6 06	5 21	7 21	3 59	8 33	3 04	9 33	3 16	9 31	4 21	8 26	5 33	6 58	6 42	5 28	7 59	4 06	9 04	3 18
10	9 09	3 44	8 08	4 59	6 50	6 09	5 19	7 23	3 56	8 35	3 03	9 34	3 18	9 29	4 23	8 23	5 35	6 55	6 45	5 26	8 01	4 03	9 05	3 18
11	9 08	3 46	8 06	5 01	6 48	6 11	5 16	7 25	3 54	8 38	3 02	9 35	3 19	9 28	4 25	8 21	5 37	6 52	6 47	5 23	8 04	4 01	9 07	3 17
12	9 07	3 48	8 03	5 04	6 45	6 14	5 13	7 28	3 52	8 40	3 01	9 36	3 21	9 26	4 28	8 18	5 40	6 49	6 49	5 20	8 06	3 59	9 08	3 17
13	9 06	3 50	8 01	5 06	6 42	6 16	5 10	7 30	3 49	8 42	3 01	9 37	3 23	9 25	4 30	8 15	5 42	6 46	6 52	5 17	8 09	3 57	9 09	3 17
14	9 04	3 52	7 58	5 09	6 39	6 18	5 07	7 33	3 47	8 45	3 00	9 38	3 24	9 23	4 32	8 13	5 44	6 43	6 54	5 14	8 11	3 54	9 10	3 16
15	9 03	3 54	7 55	5 11	6 36	6 21	5 04	7 35	3 45	8 47	3 00	9 38	3 26	9 22	4 35	8 10	5 47	6 40	6 57	5 11	8 14	3 52	9 11	3 16
16	9 01	3 56	7 53	5 14	6 33	6 23	5 01	7 37	3 42	8 49	3 00	9 39	3 28	9 20	4 37	8 07	5 49	6 37	6 59	5 08	8 16	3 50	9 12	3 16
17	9 00	3 59	7 50	5 16	6 30	6 26	4 58	7 40	3 40	8 51	3 00	9 40	3 30	9 18	4 39	8 04	5 51	6 34	7 01	5 06	8 18	3 48	9 13	3 16
18	8 58	4 01	7 47	5 19	6 27	6 28	4 55	7 42	3 38	8 54	2 59	9 40	3 32	9 16	4 42	8 02	5 53	6 31	7 04	5 03	8 21	3 46	9 14	3 16
19	8 56	4 03	7 45	5 22	6 24	6 30	4 53	7 45	3 36	8 56	2 59	9 41	3 34	9 14	4 44	7 59	5 56	6 28	7 06	5 00	8 23	3 44	9 15	3 17
20	8 55	4 05	7 42	5 24	6 21	6 33	4 50	7 47	3 34	8 58	2 59	9 41	3 36	9 13	4 46	7 56	5 58	6 25	7 09	4 57	8 26	3 42	9 16	3 17
21	8 53	4 08	7 39	5 27	6 18	6 35	4 47	7 50	3 32	9 00	3 00	9 41	3 38	9 11	4 49	7 53	6 00	6 22	7 11	4 54	8 28	3 41	9 16	3 17
22	8 51	4 10	7 36	5 29	6 15	6 38	4 44	7 52	3 30	9 02	3 00	9 41	3 40	9 09	4 51	7 50	6 03	6 19	7 14	4 52	8 30	3 39	9 17	3 18
23	8 49	4 13	7 34	5 32	6 12	6 40	4 41	7 54	3 28	9 04	3 00	9 41	3 42	9 06	4 53	7 47	6 05	6 16	7 16	4 49	8 33	3 37	9 17	3 19
24	8 47	4 15	7 31	5 34	6 09	6 42	4 38	7 57	3 26	9 06	3 01	9 41	3 44	9 04	4 56	7 45	6 07	6 13	7 19	4 46	8 35	3 35	9 17	3 19
25	8 45	4 18	7 28	5 37	6 06	6 45	4 36	7 59	3 24	9 08	3 01	9 41	3 46	9 02	4 58	7 42	6 10	6 10	7 21	4 43	8 37	3 34	9 18	3 20
26	8 43	4 20	7 25	5 39	6 03	6 47	4 33	8 02	3 22	9 10	3 02	9 41	3 49	9 00	5 00	7 39	6 12	6 07	7 24	4 41	8 39	3 32	9 18	3 21
27	8 41	4 23	7 22	5 42	6 00	6 50	4 30	8 04	3 20	9 12	3 02	9 41	3 51	8 58	5 03	7 36	6 14	6 04	7 26	4 38	8 41	3 31	9 18	3 22
28	8 39	4 25	7 19	5 44	5 57	6 52	4 27	8 06	3 19	9 14	3 03	9 40	3 53	8 56	5 05	7 33	6 16	6 01	7 29	4 35	8 44	3 29	9 18	3 23
29	8 37	4 28	7 18	5 46	5 54	6 54	4 25	8 09	3 17	9 16	3 04	9 40	3 55	8 53	5 07	7 30	6 19	5 58	7 31	4 33	8 46	3 28	9 18	3 24
30	8 35	4 30			5 51	6 57	4 22	8 11	3 16	9 18	3 05	9 39	3 57	8 51	5 10	7 27	6 21	5 55	7 34	4 30	8 48	3 27	9 18	3 25
31	8 32	4 33			5 48	6 59			3 14	9 20			4 00	8 49	5 12	7 24			7 36	4 28			9 17	3 26

Add one hour for Daylight Saving Time if and when in use.

I certify that the above data are the result of an accurate and true computation by the Nautical Almanac Office, United States Naval Observatory, an agency charged by Federal Statute (9 Stat. L 374, 375) with the duty of making such computations and publishing the results.

E. W. WOOLARD
Director Nautical Almanac
U. S. Naval Observatory