Regional Operational Plan No. ROP.CF.4K.2024.07

Kodiak Island Limnology Laboratory Analysis Operational Plan, 2024–2026

by

Sophie A. Weaver

May 2024

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	\$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	log_2, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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This document should be cited as follows:
Weaver, S. A. 2024. Kodiak Island Limnology Laboratory analysis operational plan, 2024–2026. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Operational Plan No. ROP.CF.4K.2024.07, Kodiak.

SIGNATURE PAGE

Project Title:	Kodiak Island Limnology Laboratory analysis operational plan, 2024–2026
Project Leader(s):	*Sophie A. Weaver, Fishery Biologist 2*
Division, Region, and Area:	Division of Commercial Fisheries, Region IV, Kodiak
Project Nomenclature:	
Period Covered:	May 1, 2024–December 30, 2026
Field Dates:	May 1– September 30
Plan Type:	Category I

Approval

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ABSTRACT

The Lake Assessment Project for Kodiak and Afognak islands was started in the mid-1980s as part of a comprehensive plan to assess the region's sockeye salmon *Oncorhynchus nerka* production potential. As part of the Kodiak Regional Comprehensive Salmon Plan, limnological and fishery investigations were conducted simultaneously to determine the appropriate enhancement or rehabilitation strategies for depressed sockeye salmon stocks and the stocking potential of barriered lakes without anadromous fish. The Alaska Department of Fish and Game Near Island Laboratory (NIL) was established in 2000 and has collected, processed, and analyzed limnological samples to meet these goals. In 2013, NIL was renamed the Kodiak Island Laboratory (KIL) and became Kodiak Island Limnology Laboratory (KILL) in 2017. This operational plan is formatted for functional use as a laboratory manual. It outlines the procedures used to process limnological samples for components of nitrogen, phosphorus, silicon, and photosynthetic pigments, as well as zooplankton population characteristics.

Keywords: limnology, sockeye salmon, Kodiak Island, Afognak Island, zooplankton, nutrient analyses

INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) began sampling Kodiak Island lakes for limnological data in the mid-1980s (Schrof et al. 2000). Prior to 2000, water and zooplankton samples from across the Westward Region were collected by the ADF&G Kodiak staff, processed, and shipped to the ADF&G Division of Commercial Fisheries Central Region Limnology Laboratory (CRLL) for analysis. In 2000, the ADF&G CRLL staff discontinued contract laboratory services for the Kodiak area. Westward Region staff responded by creating the Near Island Laboratory (NIL) to facilitate the collection, processing, and analyses of water samples vital to local projects. In 2013, the ADF&G NIL relocated to the new ADF&G building and was renamed the Kodiak Island Laboratory. In 2017, the lab became the Kodiak Island Limnology Laboratory (KILL) to emphasize its limnological specialization. Since the KILL's inception, the lab has collected and processed limnological samples from 72 lakes across Alaska.

The KILL processes and analyzes water samples for the following water chemistry parameters: total phosphorus (TP), total Kjeldahl nitrogen (TKN), total filterable phosphorus (TFP), filterable reactive phosphorus (FRP), total ammonia (TA), nitrate + nitrite (N+N), reactive silicon (Si), color, chlorophyll *a* (chl *a*), phaeophytin *a* (phaeo *a*), pH, and alkalinity. Historically, turbidity, calcium, carbon, magnesium, and iron have also been assessed. Zooplankton samples are processed and analyzed for species or genus abundance, biomass, and size.

Water samples analyzed by ADF&G laboratories generally employ methods from Koenings et al. (1987). However, laboratory methods are regularly updated to reflect new techniques and laboratory equipment (ADF&G 2002; Thomsen 2008; Hopkins 2017; Finkle 2020). The KILL acquired a SEAL AutoAnalyzer 3 (AA3) in 2012, and all lake nutrient concentrations, except for TKN, have been measured colorimetrically by the AA3 since 2013. TKN was contracted to South Dakota University from 2003 to 2010 and to the University of Georgia Feed and Environmental Water Laboratory from 2011 to 2017. In 2018, the KILL began processing TKN on-site. In 2019, the KILL also acquired an Orion Star T910 titrator for automated alkalinity analysis.

In addition to limnological sample collection and processing, in 2014 the KILL collaborated with U.S. Fish and Wildlife (USFWS), Sun'aq Tribe of Kodiak, and Kodiak Regional Aquaculture Association (KRAA) to establish the Water Temperature Monitoring Network for the Kodiak Island Archipelago (Pyle et al., 2014). This network monitors air and water temperature in 21 reference locations across the archipelago. The KILL and Commercial Fisheries management staff deploy, maintain, and retrieve a subset of temperature loggers annually (Pyle et al. 2014).

GOALS

1. Assess the chemical, biological, and physical characteristics of lake ecosystems to help biologists assess rearing habitat for juvenile sockeye salmon.
2. Provide sampling logistics and laboratory support for limnology data collection in the Kodiak area and Alaska Peninsula and assist ADF&G programs in other regions with limnological sample processing if capacity allows.

OBJECTIVES

1. Estimate seasonal mean lake water chemistry parameters (pH, alkalinity, and nutrient and photosynthetic pigment concentrations).
2. Quantify and measure each species or genus of lake zooplankton to estimate the seasonal mean density, biomass, and size.
3. Prepare phytoplankton samples for algal abundance and species composition analysis.
4. Collect light attenuation data to estimate lake euphotic zone depth.
5. Determine lake temperature and dissolved oxygen (DO) regimes.
6. Deploy, maintain, and retrieve air and water temperature data loggers throughout the archipelago.

TASKS

The objectives above are met with these tasks:

1. Collect lake water samples.
2. Collect zooplankton samples.
3. Preserve phytoplankton samples.
4. Collect light attenuation depth profiles.
5. Measure lake dissolved oxygen (DO; mg/L) and temperature (°C) depth profiles.
6. Measure lake water clarity with a Secchi disk (m).
7. Measure in-situ pH at the lake surface.
8. Process and analyze all collected water and zooplankton samples.
9. Process and analyze water and/or zooplankton samples from any additional subcontracts.
10. Process, package, and ship phytoplankton samples for analysis.
11. Enter, review, and archive all data into established formats (all data collected by ADF&G are available to the public upon specific request).
12. Adhere to the Department's Standard Operating Procedures (SOP), with emphasis on laboratory, aircraft, and boating safety.

GENERAL LABORATORY PROCEDURES

TRAINING

All new employees must undergo a mandatory laboratory orientation and safety training before working in the KILL. Personnel must be informed of all relevant health and safety hazards and must agree to adhere to all safety protocols. The laboratory director will instruct employees on proper laboratory technique, etiquette, relevant methodologies, safety, and quality control (QC). All continuing employees will meet annually with the laboratory director to review laboratory protocols and training materials (V:\KodiakIslandLimnoLab\Training). Additional field training opportunities will be available at the start of each field season. Current CPR and first aid certifications are mandatory. Suggested field and lab trainings include:

- Interagency Aviation Training (IAT) Water Ditching and Survival
- Occupational Safety and Health Administration (OSHA) 8-hr Hazardous Waste Operations and Emergency Response (HAZWOPER) training
- Fixed Wing Water Egress Training (US Coast Guard)

LABORATORY SAFETY

The goal of safety training is to reduce the exposure to hazards and have a plan prepared should accidents occur. KILL employees are at high risk of exposure to chemical hazards. The following objectives should be continuously referenced and implemented to mitigate risks associated with chemical hazards:

- Maintain Safety Data Sheets (SDSs) for each chemical in the facility.
- Make SDSs accessible to employees.
- Notify the Facility Safety Officer when new chemicals are brought on site.
- Train employees on how to read and use each SDS.
- Follow manufacturer's SDS instructions for handling hazardous chemicals.
- Train employees about the risks of each hazardous chemical present.
- Provide First Aid supplies and spill clean-up kits in areas where chemicals are stored.
- Have a written spill response plan and train employees annually.
- Train employees to handle chemical exposure events, cleanup spills, protect themselves, and properly dispose of waste chemicals.
- Proper maintenance of all safety equipment (eye wash, shower, etc.).
- Provide proper personal protective equipment (PPE) and enforce its use.
- Dispose of and store chemicals safely and securely.
- No food in the laboratory.
- Wear close-toed shoes in the laboratory.

Training includes proper chemical handling and hazard mitigation techniques. The KILL is outfitted with the necessary safety equipment for compliance with safety regulations (i.e. fume hoods, eye wash stations, shower, fire extinguisher, spill kits, and PPE). The lab director is responsible for replacing faulty PPE and reporting delinquencies to the Facility Safety Officer.

Laboratory Safety Training

All lab users must read the following documents found in the Laboratory Safety Notebook (LSN). The LSN can be found at the Safety Station in the laboratory (Room 140; Figure 1, cabinet 4; V:\KodiakIslandLimnoLab\Training\Laboratory Safety\Laboratory Safety Notebook). For additional information on safety, chemical use, and QC, reference the Standard Methods for the Examination of Water and Wastewater, 20th Edition (Eaton, 1998). The contents of the LSN are listed below by section:

Section 1 – KILL Laboratory Safety Training Outline

Section 2 – The State of Alaska Laboratory Safety Standard Operating Procedure 111–760

Section 3 – Kodiak ADF&Gs Hazard Communication and Chemical Hygiene Program

Section 4 – KILL Lab Safety and Hazard Communication Plan, OSHA Brief

Section 5 – KILL Safety Map

Section 6 – KILL Spill Prevention and Response Plan

Section 7 – SDS Information and Glossary of Terms

Section 8 – Wet Lab User Policy

Section 9 – KILL Laboratory Safety Training Checklist and Log

After reviewing the LSN and completing safety training, all personnel must sign the Chemistry Lab Safety Orientation Checklist (LSN, section 9). In doing so, lab personnel agree henceforth to abide by the following safety guidelines:

1. Always reference the SDS prior to using a new chemical or whenever there is a spill or exposure. Follow all SDS procedures. SDSs are on file in the laboratory (Figure 1, cabinet 4).
2. Always wear proper PPE when handling chemicals, and work in the fume hood when possible.
3. Never address a chemical spill you are not qualified to handle. Instead, immediately notify the appropriate responsible persons.
4. Always wear proper PPE and reference the Spill Response Plan prior to addressing a chemical spill, if qualified. When treating chemical exposure, always consult the SDS and reference the KILL Lab Safety and Hazard Communication Plan (LSN, section 4).
5. All chemicals should be stored in the Chemical Storage Cabinets (CSC) in the lab (Figure 1). Cabinets 21–26 are fireproof and are labeled based on content's chemical hazard (flammable, corrosive). Cabinets 27 and 28 are for non-flammable, non-corrosive chemical storage only.
6. All chemicals transferred into secondary containers must be labeled with Hazard Communication Standard pictograms.
7. Notify the lab manager or Facility Safety Officer immediately if any piece of safety equipment appears broken or an inspection is out of date.

Deionized Water Preparation

The KILL has deionized (DI) water available through faucets within the two ventilated fume hoods and at the two lab sinks. All DI water is filtered using a MilliporeSigma Milli-Q HX 7040-7150/ HR 7060-7220. The building's facilities manager is responsible for maintaining this system and changing the cartridges. Additional DI water purification has been installed at the sink level in two locations (Figure 1; sink 1 and 2). The DI water from the Milli-Q system is fed into a Barnstead B-Pure with mixed organic bed filter cartridges. This ensures the highest quality DI water at the final dispense point. The DI water is prepared with Pretreatment (VWR catalog #26291-306) and Ultrapure (VWR catalog #26303-256) cartridges using the following methods:

1. Plug in the extension cord of the megaohm meter on the B-Pure system.
2. There is a valve above each Barnstead B-Pure system that feeds purified water from the Milli-Q system. Turn the valve on for each system as described below and allow the B-Pure system to charge with water.
   a. For sink 1: turn the red valve above the water maker to the left ~1/4 turn (marked on the side of valve).
   b. For sink 2: turn the blue valve above the water maker to the left ~1/2 turn.
3. Hold the hose in the sink and allow water to flow through the system until the megohm meter stabilizes (≥18.0 megohms). Be aware of contaminating the end of the hose; do not allow it to contact any surfaces or substances. Remove hose and acid wash when needed.
4. The B-Pure system can create 18.2 megohm DI water (Type II). If the B-Pure system is not reading ≥18.0 megohms:
   a. Check the Total Oxidizable Carbon (TOC) values upstairs in the fan room on the Milli-Q. If the TOC is >30 ppb or resistivity of water is <5.0, megohms do not use the water. Contact the facilities manager to troubleshoot the system or to replace the filters.
   b. If the Milli-Q system is operating properly, change the B-Pure filter cartridges.
   c. Record the date on the Barnstead each time the cartridges are replaced.
5. When preparing standards and reagents, use 18.2 megohm water.
6. Unplug the Barnstead power cord and close all DI water system valves when not in use.

Cleaning Labware

Great care should be taken to ensure all labware are cleaned and stored properly. Glassware should be rinsed as soon as possible to prevent adherence of residue after use. If glassware cannot be cleaned in a timely manner, it is acceptable to soak the glassware overnight in DI water until it can be washed. All new glassware should be acid washed before initial use.

All Labware

1. Always wear PPE when washing or handling laboratory glassware and plastics.
2. Acid wash glassware and plastics used for nutrient analyses or sampling. Alconox phosphate-free detergent can be used to remove stubborn residue prior to acid washing if necessary.
3. Glassware and plastics used for chl-*a* analysis and zooplankton enumeration may be washed with Alconox soap and rinsed. Do not acid wash chl-*a* labware.

4. Labware may overlap for more than one method (e.g. pipette tips and beakers). Make sure these items are properly washed, dried, and stored separately based on their function.
5. Do not use bottles that have contained formalin, ethanol, or Lugol's acetate (e.g. zooplankton, smolt, or phytoplankton sample bottles) for water sample storage.

Acid Washing

All labware used in nutrient and carbon analysis (including filtration) must be acid washed. Acid wash all glassware in the 10 L 10% hydrochloric acid (HCl) bath in fume hood 1 (Figure 1).

Acid Bath

1. Always wear gloves, protective eyewear, lab coat, and closed-toed shoes when acid washing.
2. Always acid wash in the fume hood (or otherwise strongly ventilated area if in the field).
3. Remove all single-use labels or stickers before acid washing. Sharpie can be removed with acetone prior to acid washing.
4. Prepare 10% HCl to fill the designated white polypropylene acid bath basin (Common Use Solutions; page 14). Label acid bath batch date on fume hood. Always add acid to water.
   a. Add ~9 L DI water to the acid bath basin in the fume hood.
   b. Pour 1 L of concentrated HCl into a 1 L beaker. Carefully pour HCl into the basin.
   c. Top off the acid bath with DI water to the red line (10 L).
   d. This 10% HCL solution is stable indefinitely but should be replaced monthly or when visibly contaminated.
5. Prior to acid washing, dispose of any chemical waste in the proper waste receptacle. Do not dump any hazardous materials down the drain.
6. Only acid wash pre-rinsed labware. Thoroughly rinse all labware three times with DI water, removing all residue before acid washing. If necessary, soak overnight in DI water or scrub labware to remove stubborn residue prior to acid washing.
7. Place pre-rinsed dishes in the acid bath, ensuring all surfaces are covered, and rinse three times or soak for at least five minutes.

   Washing metal: do not soak stainless steel scoopulas or any metal items in the acid bath. After acid washing, promptly rinse with DI water to minimize discoloration.
8. Remove clean items and place them in designated clean (acid washed) white polypropylene tub. Transfer the dishes to a sink and rinse three times manually with DI water or transfer to the dishwasher for rinsing (Dishwasher section, below).
9. Ensure glassware is clean as indicated by uniform sheeting of water down glassware walls. Beading of water or a broken meniscus can indicate dirty glassware.

Drying and storing dishes

1. When preparing the drying area, cut a piece of bench coat, tape it to the bench, and place drying racks on top of the bench coat. Replace the bench coat annually.
2. To dry, place clean acid washed labware in the designated "acid washed" drying area by sink 1. Dry open containers upside down. Cover labware with Kimwipes when appropriate to avoid settling dust.

3. Store labware in its proper location in a timely manner. Laboratory shelves are labeled to indicate labware function and cleanliness.

Rinsing

Chl-a labware

1. Polypropylene centrifuge tubes, pipette tips, etc.:
   a. Dump all chl-*a* waste in the acetone waste container in fume hood 1 and remove labels.
   b. Fill tubes with water and use pointed agitator to loosen sediment packed into the tip of the tube. Rinse until clean (if necessary, soak overnight in DI water).
   c. Rinse all tubes and caps three times with DI water.
   d. Place inverted in a tube rack in the "rinsed only" drying area.
2. Spectrophotometer cuvettes:
   a. Empty waste into the acetone waste container in fume hood 1. Rinse with DI water.
   b. Place two drops of cleaning solution in each cuvette and gently fill with DI water.
   c. Allow to sit for five minutes, rinse three times with DI water, and once with 90% acetone.
   d. Let cuvettes air dry in the fume hood. Store in cabinet under spectrophotometer.

Zooplankton labware

1. Zooplankton bottles and glassware:
   a. Rinse three times with tap water and hang to dry on black drying rack (behind sink 1).
   b. If needed, place bottles in DI water and soak overnight to loosen any dried material and scrub to remove any residual material.

Dishwasher

The KILL contains a Labconco SteamScrubber Glassware washer plumbed with DI water to facilitate processing of laboratory dishes. This dishwasher is used for rinsing only. Detailed operating instructions can be found in the operation manual.

Rinsing nutrient plastics and glassware (post acid wash)

1. Fill the dishwasher with acid washed labware and begin rinse cycle immediately. Do not allow dishes to sit in the dishwasher; the acid will corrode the stainless-steel interior.
   a. Select Rinse cycle and set rinse total to 4.
   b. Set DI rinse to 4.

Rinsing non-nutrient plastics and glassware (post DI rinse)

1. Place items in the dishwasher.
   a. Select rinse cycle and set rinse total to 5.
   b. Set DI rinse to 2.
2. Run a rinse only cycle to prepare dishwasher for acid washed glassware.
   a. Select Rinse cycle and set rinse total to 4.
   b. Set DI rinse to 1.

PREPARING CARBON AND ISOTOPE FILTERS

Filters used in carbon and isotope filtration are combusted to eliminate contamination. The lab's annealing oven (Thermolyne 6000 Furnace, F6028C) is located on the lab cart (Figure 1; annealing oven). The only power source with the correct capacity for this oven is in the Wet Lab (Room 138) adjacent to the KILL. Please ask the Wet Lab manager for permission to use this bench space.

1. Wear gloves when preparing carbon and isotope filters.
2. Place 10–20 47 mm Whatman GF/F filters (0.7 µm; VWR catalog #28497-958) onto a small piece of heavy-duty aluminum foil (shiny side in) using clean forceps.
3. Close the foil packet by folding all open ends over at least twice to ensure a tight seal.
4. Roll the annealing oven into the Wet Lab, place on the counter, and plug into the 220-volt outlet.
5. Place filter packets in the oven and turn on the green power switch. The oven temperature has been set to 500°C. Set a timer for two hours. After two hours, turn the oven off and crack open the door. To change settings, consult the manual (Figure 1; file drawer [FD] 1)
6. After the packets have cooled, remove filters from the oven. Store packets in a labeled Ziploc bag until ready for use.
7. Re-seal foil packets after each use to prevent contamination.

PREPARING REAGENTS AND STANDARDS

Accuracy, diligence, and cleanliness are extremely important when preparing reagents and standards. Always adhere to the following guidelines and specific instructions for each method:

1. Wear proper PPE when preparing reagents and standards.
2. Avoid strong odors or perfumes in the lab. They can contaminate reagents and samples.
3. Check the SDS, expiration date, and the date opened of each chemical prior to use. Do not use expired or visibly discolored or contaminated chemicals.
4. Use type A glassware, especially when measuring and diluting corrosive class chemicals.
5. Prepare solutions in a class A volumetric flask whenever possible.
6. When measuring wet chemicals:
    a. Pour into a flask or graduated cylinder in a fume hood (Figure 1).
    b. Fill until the bottom of the meniscus touches the measurement line at eye level.
    c. Fill slowly and incrementally when nearing the end of the pour.
    d. Measure wet chemicals using volumetric flasks or cylinders with meniscus designated for delivering or containing liquids:
        i. To Deliver (TD): use the TD meniscus when a given liquid is subsequently delivered from the container. It is designed to deliver the exact volume listed when the contents are poured, accounting for residual drops.
        ii. To Contain (TC): use the TC meniscus if the flask will contain the liquid. The flask or cylinder needs every drop to deliver the precise volume.
7. When using the analytical balance to mass dry chemicals, level it by centering the bubble in the window above the readout screen. To level, turn the foot screws on each side of the balance.

8. When measuring dry chemicals, use glass weighing funnels and metal scoopulas:
   a. Place the glass funnel on the balance and tare the balance.
   b. Use a clean scoopula to transfer the dry chemical into the glass funnel, close the door, and read the balance.
   c. After measuring the desired amount, transfer the glass funnel into the opening of the volumetric flask.
   d. Rinse the funnel into the flask with DI water.
   e. With each dry chemical, use a clean scoopula and clean glass weighing funnel.
9. When pipetting: choose the most accurate pipette for the quantity needed. The lower end of the volume range will have a higher standard deviation (SD). When making standards, always check the accuracy of the pipette (1 mL DI water = 1 g).
   a. Select the appropriate pipette.
   b. Dial downwards to select the delivery volume.
   c. Select and attach a clean pipette tip with a downward twisting motion for an air-tight seal. Do not let the tip touch anything.
   d. Pour the approximate liquid volume needed into a clean, labeled secondary container for pipetting to avoid contamination.

      Pre-rinse the container: pour in enough liquid, rinse, and dispose of the rinse in a waste container.
   e. While holding the pipette vertically, depress the plunger until resistance is felt (the first stop) and immerse the tip into the desired liquid in the secondary container.

      Pre-rinse each pipette tip: pipette the liquid and dispose of the rinse in a waste container.
   f. Slowly release the plunger and allow liquid to be completely drawn into the tip.
   g. Remove the pipette from the liquid.
   h. Touch the tip to the sidewall of the receiving container and depress the plunger until the first stop and wait two to three seconds. Depress the plunger to the second stop to expel any remaining liquid.
   i. With the plunger depressed, remove the tip by sliding it up along the container wall. Slowly release the plunger when the tip is out of the receiving container.
10. Nutrient standards for all methods should be prepared on the same day that an analysis is run.
11. Prepare standards in acid washed glass volumetric flasks. Rinse acid washed flasks six times before making standards.
12. Reagents have specific shelf lives and storage methods (Figure 2).
13. Label all standards and reagent containers with your initials and the date prepared.
14. Dispose of all excess or expired reagents and standards in the appropriately labeled waste container (Reagent Disposal, below).

COMMON USE SOLUTIONS

The solutions below are prepared regularly for basic laboratory operations. Wear proper PPE and prepare solutions in the fume hood.

10% Hydrochloric Acid (HCl) Bath

Add ~9 L DI water to the acid bath basin in the fume hood. Pour 1 L of concentrated HCl into a 1 L beaker. Carefully pour the concentrated HCl into the water in basin. Top off with DI water so that acid bath basin is full to the red line (10 L). This 10% HCL solution is stable indefinitely but should be replaced monthly or when visibly contaminated.

15% Brij-35

Use a stir bar to dissolve 15 g of Brij-35 pellets in 100 mL of DI water.

Buffered Formalin

Add ~10 pellets of sodium hydroxide (NaOH) to 1 L of concentrated formalin (37% formaldehyde) or until a pH of 7 to 8 is reached.

Lugol's Acetate

Add 10 g of potassium iodide (KI), 5 g of iodine (I), and 5 g of sodium acetate-trihydrate to 50 mL of DI water. Dissolve, then dilute with DI water to 70 mL. This solution is viable for two years.

Magnesium Carbonate ($MgCO_3$)

Add 1 g of magnesium carbonate ($MgCO_3$) for every 100 mL DI water. Shake well to ensure the solution becomes fully saturated and let settle. Decant the solution from the excess $MgCO_3$ into a squeeze bottle. This solution is stable indefinitely.

REAGENT DISPOSAL

The city of Kodiak has instructed the KILL that it cannot dispose of any hazardous reagents or common use chemicals down the drain. To be compliant with the City of Kodiak, state (ADF&G and Department of Environmental Conservation) and federal (OSHA and Environmental Protection Agency [EPA]) regulations, all chemicals must be properly labeled, contained, and stored in the chemical storage cabinets or temporarily in fume hoods. Any non-hazardous chemicals that are disposed of down the drain must be recorded in the Chemical Disposal Log. These logs are posted on all fume hoods. Consult SDS sheets for specifics and further handling. At the end of each calendar year, the logs are filed into the Chemical Disposal Log notebook in the cabinet underneath the AA3, and a new log should be posted.

There is a 5-gallon waste bucket for each nutrient chemistry method (e.g. TKN waste, TP waste). Always dispose of waste in the proper receptacle and make sure that it is properly labeled with the contents of the waste.

Large quantities of weak acid (10% HCl acid bath) can be neutralized with sodium bicarbonate and disposed of down the drain, diluted 50:1 with tap water. Use pH paper to ensure that the acid is neutralized before disposing.

All hazardous waste is disposed of each spring when the Kodiak Island Borough contracts a waste service entity (e.g. National Response Corporation Alaska, US Ecology Alaska) to remove hazardous waste off the island.

SHARPS DISPOSAL

If glassware breaks, dispose of all fragments and dust in the cardboard box next to the garbage can labeled "broken glass". Used razor blades or other small sharps may also be discarded here (Figure 1). When the box is full, tape shut, and deposit in the dumpster outside.

PRESEASON PREPARATION AND METER MAINTENANCE

The KILL outfits several remote field camps with limnological equipment to facilitate the sampling objectives for their respective projects. Lab staff should check in with the lab director to determine sampling needs and to implement changes to sampling gear as needed.

Lab staff are responsible for preparing sample bottles, filtration equipment, and any other equipment. Labeled plastic totes containing an inventory list and gear for each field camp can be found in the storage room (Room 136) or in the lab (Room 140).

All meters must be inspected each season to address any issues experienced during the previous season, and to ensure that meter and sensors are calibrated, updated, maintained, purchased, and decommissioned as necessary (V:\KodiakIslandLimnoLab\Meter Calibration and Manuals\KILL Calibration and Maintenance Record.xlsx).

Temperature Loggers

The KILL is currently responsible for maintaining temperature loggers in nine systems (Appendix A3) in the Kodiak archipelago as part of the Kodiak Continuous Temperature Monitoring Network (Mauger et al. 2014, Pyle et al. 2014). Loggers are located at active weir sites (Ayakulik, Buskin, Dog Salmon, Frazer, Karluk, and Upper Station), inactive weir sites (Paul's Bay), and monitored lakes (Afognak, Akalura, and Uganik).

Prior to the first sampling event each year, the following steps should be taken to ensure the quality and continuity of temperature logger (air/water) data for each lake and weir site. Each site has a pelican case containing the loggers, deployment equipment, and instructions for weir crews. Loggers deployed in monitored lakes are deployed during the first flight in May. For loggers that overwinter, shuttles will be used to download data from deployed loggers.

1. Check the Inventory and Deployment log spreadsheet on the network (V:\KodiakIslandLimnoLab\Hobo loggers) for notes about the season and overwintering status of each logger by location.
2. Check the Temp Monitoring Network Logger QC and Deployment notebook to make sure the logger status and serial numbers (air/water) associated with each monitoring site match the spreadsheet.
3. For temperature loggers that are returned to Kodiak at the end of the field season, use the Hobo Waterproof Shuttle to dock each temperature logger and assess the battery life.
    a. If the battery life is "good", proceed with the pre-season QC checks.
    b. If the battery life for a water temperature logger is "low", select a new logger.
    c. Return water temperature loggers with low batteries to Onset for battery replacement.

d. If the battery life for an air temperature logger is "low", replace the battery with a new ½ AA and begin QC checks.
e. Note logger status after each QC check.

4. For loggers that overwintered, prepare backup air and water temperature loggers for each weir or lake site in case the loggers do not successfully overwinter.
   a. Update the logger instructions for each site and proceed with pre-season QC checks for the new loggers.
   b. If the same logger has been successfully deployed for two years, instruct crews to replace the logger and send the old logger back to the lab for inspection and QC checks.
   c. Check paperwork for current river deployments to determine how loggers are secured (bank, in-river). Prepare a deployment kit for each crew with an appropriate length of 1/8" coated wire, C-clamps, wire clips, and lead weights for deployment.
5. When making decisions at the end of the season about whether to instruct crews to let loggers overwinter, consult with the lab director and the Inventory and Deployment log spreadsheet for notes from previous years.

Li-Cor LI-250A Light Meter and Underwater Quantum Sensor

The Underwater Quantum (UWQ) sensor will need to be calibrated once every two years. Contact Li-Cor to request a return material authorization number for recalibration. Store the UWQ sensor with the red cap on to protect the white photometer when not in use. Be careful not to pinch the meter cable when closing the case.

Calibration Instructions

Upon receiving a newly factory-calibrated UWQ sensor, input the new multiplier value into the LI-250A following the instructions below. These instructions can also be found on the back of the meter. The new calibration data and multiplier value should be laminated and attached to the light meter in case the value is deleted in the field (V:\KodiakIslandLimnoLab\Meter Calibration and Manuals\Light Meters\Calibration multiplier Tag Templates.xlsx).

1. Turn meter on, press **CAL** to view or change the active multiplier.
2. Press ↑ or ↓ to change the multiplier value.
3. Units should be entered in µmol ($s^{-1}m^{-2}$ per µA). To change units, press **UNITS** to toggle through options.

YSI Pro10 pH Meter

The pH sensor will need to be replaced every 12 to 24 months. Refer to the manual for instructions on replacing the sensor cap. If repairs are necessary, the pH meter can be sent to YSI for servicing. The pH sensor must be stored in a moist environment. For short term storage, moisten the sponge and store the instrument in the grey storage sleeve. For long term storage (i.e. >30 days), insert probe into storage bottle filled with enough pH 4 fluid to cover the sensor and cap tightly. Be careful not to pinch the meter cable in the case when closing as this can make the sensor inoperable.

Calibration Instructions

Calibration may be performed at 1-, 2-, or 3-points. Each day before sampling, perform a 3-point pH calibration.

1. Remove the grey sleeve and the metal guard and immerse the probe in pH 7 buffer, making sure the probe's temperature sensor is also below the buffer surface.
2. Press and hold **Cal** for three seconds to enter the calibration menu.
3. Highlight 3-point and press **ENTER**.
4. Wait 30 to 60 seconds for the buffer to stabilize. Once stable, press **ENTER**. Verify that the mV value is within the correct range for this pH buffer solution. If the reading is stable, the meter will display "**2nd pH calibration point**"
5. Rinse the probe in tap water, gently dry with a Kimwipe, and place it in the second buffer (pH 4 or 10).
6. Repeat steps 4–6 for pH 4 and pH 10 buffers.
7. At the end of calibration, the screen will read "**calibration successful**" and you will be returned to the original screen. Rinse the probe, replace the metal guard and grey sleeve, and have fun sampling!

YSI Professional Optical Dissolved Oxygen (ProODO) Meter

The sensor cap will need to be replaced once every two years. Refer to the manual for instructions on replacing the sensor cap. The ProODO sensor must be stored in a moist environment. For short term storage, moisten the sponge and slide the gray sleeve over the probe guard. For long term storage (i.e. >30 days), in addition to the gray calibration/transport sleeve, soak the sponge in the red protective cap and place the red cap over the probe tip. Check the sponge every 30 days to ensure it is moist. If the sensor dries out, rehydrate the sensor cap. Be careful not to pinch the meter cable in the carrying case when closing.

Calibration Instructions

The ProODO sensor is very stable relative to traditional electrochemistry sensors, but calibrate DO and barometric pressure with each use for accuracy.

DO Calibration

1. Moisten the sponge in the gray calibration/transport sleeve with clean tap water. The sponge should be clean and moistened. Make sure there are no water droplets on the optical dissolved oxygen (ODO) sensor cap and temperature sensor. If sensor is wet, dry with a Kimwipe. Place the calibration/transport sleeve over the probe. The ODO and temperature sensors are now in a humid environment.
2. Turn the instrument on and wait five minutes for the air in the calibration/transport sleeve to become fully saturated.
3. Press **CAL**, then highlight **DO** and press **ENTER**.
4. Highlight **DO%**, then press **ENTER**.
5. Verify that the barometric pressure (BP) displayed is accurate. Once DO and temperature are stable (wait at least 30 seconds), highlight **Accept Calibration** and press **ENTER**. The screen will indicate if the calibration has been accepted and the DO% should display as 100%.

Barometric Pressure Calibration

1. Determine the "true" BP in mmHg, which is BP not corrected to sea level. The lab sits at ~104 feet above sea level. The true BP is estimated with the following formula:

   True BP = Corrected BP – [2.5 x Local altitude above sea level (ft)/100]

2. Press the **CAL** hotkey on the keypad.
3. Highlight **Barometer** and press Enter. Use the arrow keys to highlight the desired units (mmHg) and press Enter.
4. Highlight **Calibration Value** and press Enter.
5. Use the Alpha/Numeric screen to enter the true BP, then highlight **Enter** and press the Enter key. Highlight **Accept Calibration** and press **Enter** to finish the calibration.

LABORATORY EQUIPMENT SERVICE AND MAINTENANCE

Orion Star T910 pH Titrator

In 2019, the KILL acquired an Orion Star T910 pH Titrator to automate the lab's alkalinity titrations. The T910 is equipped with a stirring probe, Automatic Temperature Compensating (ATC) probe, and a ROSS Ultra electrode. We use this titrator to measure both pH and alkalinity. Detailed maintenance instructions for each component of the T910 can be found in the in the lab (Figure 1) and on the network (V:\KodiakIslandLimnoLab\Meter Calibration and Manuals\ Autotitrator). The ROSS Ultra electrode lifespan is 1 to 2 years. If the electrode is taking an excessive amount of time to measure pH (>5 mins) or alkalinity (>30 mins), replace the electrode.

Maintenance Schedule

Daily Maintenance

1. Wipe off chemical spills immediately to avoid damage to the case.
2. Keep the titrator dry, do not allow liquid to penetrate the computer housing.
3. Check for air bubbles in the line and burette. Purge all bubbles prior to use.
4. Inspect the electrode for scratches, cracks, salt buildup or membrane/junction deposits. Rinse off any salt buildup with distilled water.
5. Short-term storage (<1 week): fill the reference chamber with fill solution (VWR, catalog # 810007) and securely cover the fill hole. Place the electrode in the storage bottle and soak in storage solution (VWR, catalog #810001).
6. Long-term storage (>1 week): fill the reference chamber with fill solution and securely cover the fill hole. Cover the sensing bulb and reference junction with the protective sleeve or storage bottle and add a few drops of storage solution. Upon next use, prepare as a new electrode (instructions below).

Weekly to Monthly Maintenance

1. Drain and replace the electrode fill solution.
2. Change the storage solution in the electrode storage bottle.
3. Standardize the titrant.

Three-month Maintenance

1. Inspect the burette piston for cleanliness and a tight seal. Inspect the glass for chips or damage.
2. Inspect all seals and tubing for a tight seal. Check for damage to the fittings and tubing and check for tubing flare at the ends. Replace as needed.
3. Inspect the dispenser and ensure there are no particle blockages or leaks at the tubing connections or at the dispenser tip.

Yearly Maintenance

1. The dispenser tip should be replaced as needed but routinely done every 12 months.
2. When taking the titrator off-line for the season, follow the Titrator and Burette Storage instructions (Orion Star T900 Series User Manual; page 20, 68X700190, 2017). Follow the long-term electrode storage instructions (User Manual; page 10, 263745-001, 2014).

Electrode Preparation

After long term storage (>1 week), the electrode should be prepared as new.

1. Remove the protective sleeve or bottle from the sensing bulb.
2. Clean any salt deposits by rinsing with distilled or tap water.
3. Uncover the fill hole and fill with electrolyte fill solution to the bottom of the fill hole.
4. While in use, the fill hole should remain open and the fill solution should be above the reference junction and at least 1 inch above the sample level.
5. Suspend the electrode in the holder for five minutes to thoroughly wet the reference junction. Shake the electrode downward to remove any air bubbles.
6. Soak the electrode in ROSS pH electrode storage solution for 30 to 60 minutes.
7. Connect the electrode to the meter.

Genesys 10S VIS Spectrophotometer

The KILL uses a Genesys 10S VIS Spectrophotometer to measure sample absorbances for color, chl *a*, and phaeo *a* (Figure 1). The spectrophotometer features a 6-position cell holder with automatic alignment, graphical backlight display, and single-beam optical geometry. This spectrophotometer is capable of measuring wavelengths in the visible spectrum from 325 to 1100 nanometers (nm). This instrument should be sent in for servicing every two years.

The instrument is used in "Absorbance" mode for all measurements at the KILL. Once in absorbance mode, each absorbance measurement will have to be measured against a "blank" every time the wavelength is changed. The blank matrix should be consistent with the sample matrix (i.e. acetone blanks for chl *a*, DI water for color, etc.)

Tuttnauer Autoclave 2540E-B/L

In 2016, the KILL acquired a Tuttnauer Autoclave 2450E-B/L. Before each batch, check the water level in the reservoir and top off if needed. Water should be up to but not covering the safety valve. After each batch, the door gasket should be cleaned with water and a soft cloth to rid it of debris to prolong the gasket's life. All other maintenance measures listed in the manual can be carried out on an annual basis.

Pipettes

The lab's pipettes are professionally calibrated annually. To access documentation on each pipette's service history, log in to their website (Pipettes.com; V:\KodiakIslandLimnoLab\ KILL Vendors\Vendor Acct Info.xlsx).

AA3 Segmented Flow AutoAnalyzer

The KILL uses a fully automated nutrient analysis protocol. The KILL has a service contract with the instrument's manufacturer, SEAL Analytical, wherein annual maintenance is performed by a SEAL technician. For interim maintenance procedures refer to AA3 Maintenance Procedures (page 45).

PURCHASING

Prior to making a purchase, ensure that the vendor is known to the state. If the vendor is new, their tax identification and contact information must be provided to administration staff prior to making a purchase. All purchases made by the state are tax exempt; any taxed purchases must have the tax removed by the vendor and reinvoiced. All purchases executed online through approved vendors can be paid via invoice. If the vendor requires a purchase order number, use KILL and the 6-digit date (e.g. KILL021920). Receipts from purchases made with a One-Card should be submitted to administrative staff within three days. Tape small receipts to the "Invoice" or "P-Card" form (FD 2, Figure 1). Provide the lab director all receipts and invoices for coding and submission to accounts payable staff for payment.

Large expense purchases must be approved by the lab director prior to purchasing or submitting any paperwork to administrative staff. Any single purchase over $2500 requires a Request For Quote (RFQ) that is submitted to ADF&G Procurement by Kodiak administrative staff. With each new RFQ, make a folder and save the completed document and all associated records in this folder.

All purchases should be recorded in the KILL Purchases folder (V:\KodiakIslandLimnoLab\KILL Purchases) and in the budget tracker (V:\KodiakIslandLimnoLab\Admin and Budgets\Budgets). New subfolders should be made annually to store digital copies of paper receipts and invoices and should be formatted as follows: \KILL 20XX Purchase Receipts\Invoices. These files should be titled with the purchase date, followed by the vendor name and saved as a .pdf. A new spreadsheet should also be made annually to itemize each purchase (V:\KILL 20XX Purchase Receipts\20XX Lab purchases.xls).

SHIPPING

The account information for the lab's approved shipping and commodities vendors can be found on the network (V:\KodiakIslandLimnoLab\KILL Vendors\Vendor Acct Info.xlsx). Shipment of frozen samples should be expedited using FedEx. The U.S. Postal Service (USPS) and FedEx are acceptable for all other purposes. However, when considering a shipping method, the USPS is the preferred method if the shipment is not large nor requires expedited service.

Preparing Samples for Shipping

1. Contact the laboratory or sample recipient (Appendix A1) prior to shipping to ensure they have the capacity for the samples and the samples can be analyzed within a specified time frame.
2. Properly label samples with date, lake, station, sample depth, sample type, and preservative used if applicable.

3. Populate the appropriate chain-of-custody (COC) form and verify that all samples listed are accounted for. Print a hard copy to include with the shipment.
4. When shipping liquid samples, tape the lids with electrical tape. Pack the samples upright.
5. Protect the inventory list in a sealable plastic bag and include it in the package. An electronic copy should also be emailed to the recipient.
6. Use arrows or stickers pointing upwards to help handlers keep samples upright during transport.
7. Send all frozen water and phytoplankton sample shipments no later than Wednesday of each week to ensure safe arrival before the end of the work week.
8. Forward the package's tracking information to the recipient whenever possible, and/or notify the recipient electronically with the package's expected arrival date and time.

Receiving Samples

When receiving samples make sure to carefully review the inventory and complete any accompanying COC forms. Immediately notify the sender of any discrepancies. Store all frozen samples on the designated shelves in the –20°C walk-in freezer. Store zooplankton samples in the drawer by the microscopes (Figure 1).

WORK TRACKING AND DATA ENTRY

All sample processing needs to be tracked to ensure billing transparency and document lab efficiency for both the lab staff and director. There are two work trackers that need to be maintained as samples are processed. They track the analyses and fieldwork completed for KRAA contract lakes (V:\KodiakIslandLimnoLab\Admin and Budgets\Contract work-Limno\KRAA\20XX\ 20XX KRAA contract work tracker.xls) and all other water chemistry samples (V:\KodiakIslandLimnoLab\KILL Experiments\ ANALYSES_RUN_20XX.xls).

Following data collection or sample processing, data are entered into the Kodiak Intranet Westward Region WIKI (WIKI) database, established in 2012. The WIKI will be used to enter all chemical, physical, and biological data generated by the lab (http://kodweb.fishgame.state.ak.us/). There is a desktop computer available for data entry in the lab (Figure 1).

After each WIKI data entry event, initial and date data sheets and place in the "to be checked" bin in room 134. All entered data is reviewed. The data should be checked either by a different individual or at a different time or day by the same individual. Once data has been checked, initial, date, and file it in the appropriate data folder in the lab (Figure 1).

QUALITY ASSURANCE AND QUALITY CONTROL

To maintain high data quality standards, the KILL employs quality assurance (QA) and QC measures. Producing high quality data when sampling and running chemistries necessitates all lab staff maintain a strict attention to detail, cleanliness, and adherence to all protocols.

Quality Assurance - Process Tasks

1. Follow lab practices (General Laboratory Procedures; page 7) to ensure labware cleanliness and reagent and standard quality.
2. Follow the lab and field practices (Limnological Sampling Procedures; page 23) to ensure high quality of all chemical, physical, and biological samples and data collected.

3. Always wear gloves when handling lab glassware.
4. Use DI water from sink 2 (DI water faucet and Barnstead B-Pure) when washing glassware.
5. Use fresh 18.2 megohm DI water when preparing standards and reagents. Make new working standards for every nutrient analysis.
6. Calibrate all instruments prior to use. Use a calibration standard when available.
7. Avoid performing nutrient analyses if strong odors are present in the lab.
8. Run blanks, blind check standards, replicates, and spikes during every nutrient analysis.

Quality Control - Outcome Tasks

1. All runs of blanks, blind check standards, spikes, and other diagnostics should yield values within the range specified by the method and standard (See Run QC Criteria, page 49). If values are out of range, remake solutions or re-run the nutrient chemistry.
2. Regularly assess nutrient method parameters by maintaining control charts and regularly assessing detection limits. Lab practices are evaluated based on the results of these metrics.
3. Regularly participate in external inter-laboratory comparisons such as the Standard Reference Sample Project run by the U.S. Geological Survey (USGS, http://bqs.usgs.gov/srs). Performance should be within the ranges recommended by the USGS.
4. After each WIKI data entry event, check data. The data should either be checked by a different individual or at a different time or day by the same individual.
5. When compiling data reports from the WIKI, check biomass and density calculations for correctness. Report all issues with the WIKI Limnology Database to the staff Data Analyst/Programmer and lab director.

LIMNOLOGICAL SAMPLING PROCEDURES

Limnological sampling occurs once a month from May through September and is generally scheduled for the middle of each month. Sampling occurs from the float of a float plane, and the pilot will perform safety training upon field sampling. IAT Water Ditching and Survival training is available for KILL employees through the USFWS. Field gear should consist of waders, bear spray, a PFD, and survival kit. Gloves and a personal Garmin inReach device or communications device are recommended but not mandatory.

A typical sampling event at a single station consists of collecting a 6 L water sample, 100 mL zooplankton sample, Secchi depth, 1 m pH, and vertical profiles of light attenuation, DO, and temperature. Sampling should not occur if the lake surface is choppy or wind (>10 knots) causes sampling gear to pay out at greater than a 10° angle as the sample will not be collected at an accurate depth. The airplane pilot often helps with sampling and recording duties.

PRE-FLIGHT PREPARATION

1. Consult the *Field Sampling Equipment List* (Appendix A; Figure 1; cabinet 9). Assemble all the required sampling gear.
2. Prior to going out on the first flight of the season, double check the length of all spools of line. Knowing the line length is critical for setting stations effectively and safely.
3. Check that the metered cords for each sensor are properly taped with correct depth increments labeled (i.e. DO and temperature to 50 m, light attenuation to 30 m, pH at 1 m)
4. Consult the limnology sampling and processing schedule (e.g., Weaver, 2022) to verify which lakes, stations, and depths are to be sampled.
5. Obtain a clearly marked morphometric map showing global positioning system (GPS) station locations by latitude and longitude at the lake being sampled (Figure 1; FD 2; V:\Kodiak IslandLimnoLab\Lake_Maps and GPS Coordinates).
6. Acid wash the 8 L plastic carboys needed for sampling. The lids of the carboys are pre-labeled with the lake name, station, and sample depth.
7. Prepare the 125 mL polypropylene zooplankton bottles.
   a. Label bottle lids and labels with the lake names and stations. Record the date and tow depth while sampling.
   b. Add 10 mL buffered formalin (10% of total volume while accounting for filling bottle to shoulder) to each polypropylene bottle and cap tightly.
8. Prepare chl-*a* petri dishes, amber phytoplankton bottles (100 mL), unfiltered water sample bottles (2 x 250 mL), and filtrate sample bottles (500 mL) by labeling with lake name, station, and depths sampled. Date the sample bottles after returning from a successful sampling trip.

LAKE STATION SET-UP

1. Locate the appropriate station location using a GPS and lake map.
2. Verify the position of sampling station. Lake stations are left in place over winter and ice may move the stations, requiring them to be reset. If a station is missing, set a new station:
   a. Fill three sandbags with gravel on the lakeshore, preferably near the station.

b. While on shore, place a ~ 4’x4’ section of netting on the floats near the rear of the plane. Center the sandbags on the netting. Unspool enough of the ¼” polypropylene line to tie the sandbags together and stitch them into the netting. Tie the stacked sandbags together with the running end of the station anchor line. Securely wrap the tied sandbags with the netting and stitch the netting closed. Secure the running end of the line to the standing end of the line closest to the sandbags.
c. Cleat the sandbags off with the standing end of the station anchor line, get back in the plane, and the pilot will slowly taxi to the GPS buoy station location.
d. Upon nearing the buoy station, insert a metal pipe through the spool of polypropylene line. Be aware of hang-ups and make sure everything and everyone is clear of the line prior to dropping bags to the lake bottom.
e. Double check the lake depth: make sure you have enough line.
f. When the person holding the pipe and spool of line is ready, a second person will un-cleat the line and shove the pursed sandbags off the edge of the plane float as close to the GPS buoy site as possible. The sandbags will sink to the lake bottom.
g. Attach a 3-cork buoy system leaving 3–4 m of scope in the line. This reduces drag during a sampling event and accommodates fluctuations in lake depth. During summer sampling months, attach an orange polyform buoy (size A2 or A3) for ease of locating sampling stations. Leave only the 3-cork buoy system to overwinter.

WATER SAMPLING

Collect water samples at designated lake stations using a 4 L or 6 L Van Dorn sampler.

1. Set up the Van Dorn in an open position. Attach the messenger to the line.
2. Lower the Van Dorn to 1 m. The line should be completely vertical in the water column.
3. Send the weighted messenger down the rope to trigger the release mechanism that closes the Van Dorn.
4. Pull the Van Dorn to the surface. Rinse the carboy with ~ 0.5 L of sample water and discard the rinse water. Rinsing removes any DI water left in the carboy. Pour the remaining sample water into the carboy. Ensure that the sample is not contaminated.
5. Repeat this procedure until the carboy contains ≥4 L of sample water. When finished, screw the cap on tightly and place the carboy in the airplane floats as directed by the pilot.
6. Record sampling depths, stations, and other appropriate comments on the *Detailed Lake Survey Form* (DLS; Figure 3).

ZOOPLANKTON SAMPLING

Collect zooplankton samples at all lake stations using vertical tows with a 0.2 m diameter, 153 µm mesh conical net.

1. Verify the tow depth. For lakes <50 m deep, measure the station depth by lowering a weighted, metered line to the lake bottom. For lakes >50 m depth, zooplankton tow depth is 50 m.
2. Calculate zooplankton tow depth. The zooplankton net is 1 m long, therefore:

   Tow depth = measured depth – 1 m.

3. Lower the net into the lake at a steady rate (so that the plankton bucket stays below the net opening) until you reach the tow depth.
4. Retrieve the net manually, at a strictly vertical angle, at a constant rate of 0.5 m/sec or faster. Stop when the net rim is above the lake surface. Once at the surface, slowly work the contents of the tow net towards the plankton bucket without re-submerging the net rim.
5. Rinse the net by lowering it into the lake two to three times while keeping the net rim above the surface. If zooplankton is visible on the net, rinse from the outside with a DI water squirt bottle starting at the top and working down to the cod end to wash the zooplankton stuck on the net into the plankton bucket.
6. Remove the plankton bucket from the net, decant excess water through the mesh screen, and pour the contents into the appropriately labeled 125 mL polypropylene bottle containing 10 mL buffered formalin.
7. Rinse the plankton bucket screen with the DI squirt bottle and pour the contents into the polypropylene bottle. Repeat until all zooplankton are transferred to the sample bottle, and the sample bottle is full, achieving a 10% preservative solution.
8. Seal the bottle cap with electrical tape for transport and storage.
9. Record the tow depth on the DLS form and write the tow date, depth, and sampler's initials on the zooplankton sample bottle.
10. At each lake, rinse the net and plankton bucket in the lake before sampling. Upon returning to the lab, thoroughly rinse the net and bucket with tap water and air dry.

Light Attenuation Measurements

The KILL uses a Li-Cor LI-250A light meter and LI-192 Underwater Quantum (UWQ) sensor to measure light attenuation at all lake stations. The KILL only measures downwelling irradiance.

1. Measurements are taken on the plane or boat's sunny side to avoid shading the sensor and creating variable readings. If cloud cover varies, light intensity readings will be inconsistent; wait for the cloud cover to stabilize before taking measurements.
2. Before sampling, check that the calibration coefficient matches the "in water" calibration coefficient attached to the UWQ sensor (units in µmol). If they do not match, adjust the calibration coefficient by pressing **CAL** on the meter and toggling to the correct value.
3. Measure light readings, or photosynthetically active radiation (PAR), just below the surface, to a depth of 5 m at 0.5 m intervals (i.e., 0.5, 1.0, 1.5, …., 5.0 m), and then every meter (6.0, 7.0, 8.0 m, etc.) until the light is at least 1% of the surface reading or the bottom is reached.
4. Record the measurements at each depth on the DLS form (Figure 3).
5. Upon returning to the lab, open the case and allow the meter to air dry. Store the meter with the red cap in place to protect the sensor.

Temperature and Dissolved Oxygen Measurements

Measure water temperature (ºC) and DO (mg/L) concentrations at all lake stations with a YSI ProODO meter.

1. Examine and test the sensor cap on the meter probe prior to use. Replace the sensor cap if there is physical damage or the meter readings appear inaccurate.

2. Calibrate the ProODO for barometric pressure and water saturated air (DO %) prior to taking measurements. Perform these calibrations on the day of sampling, and whenever the sensor cap is replaced or rehydrated (DO Calibration, page 17).
3. To measure DO and temperature, remove the grey sleeve and red cap from the sensor, but keep the metal guard in place. Lower the probe into the lake. Tilt the probe side to side to release any air bubbles trapped on the probe.
4. Measure temperature and DO from the surface to a depth of 5 m at 0.5 m intervals (i.e., 0.5, 1.0, 1.5, …., 5.0 m), and then take measurements every meter (6.0, 7.0, 8.0 m, etc.) until 25 m and then every 5 m until 50 m or the lake bottom is reached.
5. Record the data on the DLS form (Figure 3).
6. If done sampling for the month, replace the red cap on the DO sensor. If sampling more lakes within the month, leave the red cap off but replace the grey sleeve. There should be a clean, wet sponge in the sleeve to keep the sensor moist.
7. Upon return from the field, open the case and allow the meter to air dry.

pH MEASUREMENTS

Calibrate the pH meter in the lab before measuring pH in the field (pH Calibration, page 16). After each sampling event, pH and alkalinity will also be measured in the lab.

1. Measure and record pH at 1 m at all lake stations with a YSI Pro10 Hand-held pH meter.
   a. Insert the probe 1 m into the lake, wiggle cord gently to remove any trapped air bubbles and wait for the readings to stabilize (~5 minutes).
   b. Record the data on the DLS form.
2. If collecting an in-situ sub-surface water sample, allocate water to measure pH.
   a. Pour water from the Van Dorn or storage carboy into a 500 mL bottle.
   b. Submerge the pH meter into the sample, remove any trapped air, and wait for the reading to stabilize.
   c. Record the data on the DLS form.

SECCHI DISK DEPTH

Measure and record the Secchi disk depth at all lake stations as a measure of water clarity. Secchi disk depth should be measured on the shaded side of the boat or airplane or taken underneath the wing. Remove polarized glasses before measuring Secchi disk depth.

1. Lower the Secchi disk into the water on a metered line and note the depth the disk disappears.
2. Slowly retrieve the Secchi disk and note when the disk reappears.
3. Record the depths of disappearance, reappearance, and the average of the two readings on the DLS form (Figure 3).

WATER SAMPLE PROCESSING PROCEDURES

After returning from a sampling trip, all water sample carboys should be refrigerated immediately and kept in the dark. Water sample processing procedures (Appendix A4) should be completed within 72 hours of collection, preferably within the first 24 hours. Particulates in the sample will settle over time and need to be resuspended prior to processing. Invert and shake the carboy to ensure the sample water is well mixed. Label bottles ahead of time with lake name, station, and sample depth for more efficient processing.

After a sampling event, there will be:

- 1 x 125 mL zooplankton sample (collected in the field)
- 1 x 500 mL filtered water sample
- 2 x 250 mL unfiltered water sample
- 1 x 500 mL unfiltered water sample (for immediate pH and alkalinity measurements)
- 1 x 125 mL phytoplankton sample
- 1 carbon filter sample
- 1 chl-*a* filter sample

UNFILTERED WATER SAMPLE PROCEDURE

Rinse each water sample bottle with a small amount of its respective sample water prior to filling to remove any residual DI water. Write the collection date on the label after sample collection.

1. Unfiltered refrigerated sample (pH and alkalinity):

   If measuring pH and alkalinity immediately, transfer 200 mL of sample into a 250 mL beaker. If measuring pH and alkalinity within 72 hours, completely fill and label a 500 mL polypropylene bottle. Cap and refrigerate.

2. Unfiltered frozen sample (TP, TKN, Si):

   Fill two 250 mL polypropylene bottles with ~200 mL of sample and cap. Leave space at the top to allow for expansion from freezing. Label one bottle 1 of 2, and the other 2 of 2. Freeze immediately in the large walk-in freezer (–20° C) on a limnology shelf (Figure 1).

3. Phytoplankton sample:

   Using a graduated cylinder, measure 100 mL of sample water and pour into an amber 125 mL polypropylene bottle. Add 2 mL Lugol's acetate (Common Use Solutions; page 10), mix gently, and seal the bottle with electrical tape. Store in a labeled box in the dark at room temperature.

FILTERED WATER SAMPLE PROCEDURES

Processing filtered water samples yields filtered water and particulates samples. Particulates are collected on two separate filters: one for chl-*a* analysis and one for carbon analysis. Filtered water is used for nutrient analyses and is processed using pre-combusted filters and an acid washed filtration flask and funnel. Filters and filtrate should be frozen and stored in the –20° C walk-in freezer until analysis.

Equipment

Whatman GF/F filters (47 mm)
Filter petri dishes
Chl-*a* forceps
Nutrient forceps
Graduated cylinders
DI water squeeze bottle
Filtration apparatus (Figure 4)
Magnesium carbonate solution in squirt bottle
500 mL polypropylene bottles
Kimwipes

Preparing Filtration Equipment

1. Acid wash all nutrient filtration equipment (i.e. filter funnels, flasks), graduated cylinders, and forceps prior to filtering water samples. Filtration equipment used for chl *a* will not be acid washed, just rinsed.
2. Set up the two-pump vacuum filtration assembly (Figure 4).
3. Apparatus A is designated for nutrient and particulate carbon samples. Attach the vacuum pump labeled with red tape to the filtration manifold labeled with red tape; attach three filtration flasks to each hose of the manifold, and insert the three filter funnels seated with black, number 9 rubber stoppers. Set pump suction at 4 psi.
4. Apparatus B is designated for chl *a.* Attach the vacuum pump labeled with blue tape to the waste carboy. Attach the filtration manifold also labeled with blue tape and insert the three filtration funnels seated with number two rubber stoppers. Set pump suction at 4 psi.
5. Filter in darkness to minimize production by phytoplankton.

Collecting Particulates – Chlorophyll *a* and Carbon

1. Remove the magnetic cups from the filter funnels. With sterile forceps, place a Whatman GF/F filter on each of the chl-*a* filter funnel bases (apparatus B). Using a second set of sterile forceps, place one pre-combusted Whatman GF/F filter on each carbon filter funnel base (apparatus A).
2. Choose a water sample. Rinse the two clean graduated cylinders labeled “1” with ~200 mL of sample water. Two sets of graduated cylinders are numbered 1 through 3; the glass cylinders are for particulate carbon/filtrate (apparatus A) and the plastic cylinders are for chl *a* (apparatus B). The cylinder should always match the filter cup.
3. Fill the rinsed graduated cylinders labeled “1” with 1L of sample water and place them in front of position 1 on both apparatus A and B.

4. Repeat steps 3 and 4 for subsequent water samples using all three positions on the filtration manifold. Be consistent and keep track of samples.
5. Begin filtration for particulate carbon (apparatus A):
    a. Pour ~150 mL of sample water into each of the three corresponding filter cups. Turn on the pump and draw the water into the filtration flasks. Turn off the pump, swirl to rinse, and discard filtrate. This rinses any remaining DI water from the flasks.
    b. Turn pump back on and filter the remaining ~900 mL by pouring water from the designated graduated cylinder into the corresponding filter cups.
    c. Turn off the pump when all water has been drawn through the filter. The filtered water in the flask is the filtered frozen sample.
    d. Remove the magnetic cup from the base. Using sterile forceps, fold the filter in half, and place in a particulate petri dish labeled "carbon". Store in the dark in the walk-in freezer.
6. Begin filtration for chl *a* (apparatus B):
    a. Fill each filtration cup with sample water from the corresponding graduated cylinders. Turn the pump on and continue drawing water through until ~950 mL has been processed.
    b. When ~50 mL of the sample remains in the chl-*a* filter funnels, add ~5 mL (1 squirt from the squirt bottle) of $MgCO_3$ solution to the sample (Common Use Solutions; page 14). Rinse down the sides of the filter funnels with DI water to ensure that all the particulates are collected on the filters.
    c. Turn the pump off when all the water has been drawn through the filter.
    d. Remove the magnetic cup from the base. Using chl-*a* forceps, fold the filter in half, place in a labeled particulate petri dish labeled "chl *a*", and store in the walk-in freezer.
    e. After all samples have been filtered, filter a QC sample called a "filter blank". Perform steps a through d by filtering 1 L of DI water.
    f. Detach the pump and run for five minutes.
7. Filtration should take less than 10 minutes. If filtration appears sluggish after ~8–9 minutes, do not pour any more water in the filter funnel. Finish filtering what's in the funnel, subtract the water volume remaining in the graduated cylinder from the original 1 L sample, and record the actual volume filtered on the petri dish.

Filtered Frozen Water Sample

Filtered frozen sample bottle (color, FRP, TFP, N+N, TA): Prepare a labeled 500 mL polypropylene bottle by rinsing with ~50 mL of filtrate. Swirl and discard. Pour ~ 450 mL of filtered water into the polypropylene bottle, allowing room for expansion as the water freezes. Freeze immediately and store in the large walk-in freezer on a designated limnology shelf.

ZOOPLANKTON SAMPLE PROCESSING AND ANALYSIS

Zooplankton are the primary food source for juvenile sockeye salmon. Zooplankton community structure is affected seasonally by predation from salmon fry and changing environmental conditions (Lind 1985). Monitoring changes in zooplankton community structure provides information that can be used to evaluate rearing conditions and assess the stocking and enrichment program results (Koenings et al. 1987). Most agencies that the KILL serves have a vested interest in salmon rearing, stocking, or nutrient enrichment; zooplankton analysis is an important service provided by the KILL.

Equipment

- Microscope with ocular micrometer
- Sedgwick-Rafter counting slide (S-R slide)
- Zooplankton Data Sheet (Figure 5)
- Henson-Stemple pipet
- Funnel
- Formalin waste bottle and bucket
- PVC sieve (153 µm mesh)
- DI water squirt bottle
- Magnetic stirrer
- Stir bar
- Beakers
- Kimwipes
- Wildco Zooplankton splitter

Chemical Handling and Disposal

Formalin is a health hazard. Wear gloves and avoid contact or inhaling fumes. Consult SDS for specifics. Dispose of all formalin waste in the labeled waste bucket in the fume hood.

SAMPLE PREPARATION

1. Place the funnel in the formalin waste bottle. Place the PVC zooplankton sieve so it is positioned upright and square within the funnel.
2. Empty the zooplankton sample bottle contents into the sieve, rinsing the zooplankton sample bottle and cap three times with the DI water squirt bottle to remove all zooplankton.
3. Gently tilt the sieve to one side and rinse the zooplankton from the mesh and sides of the sieve so it is concentrated at the lowest point on the sieve.
4. Carefully transfer the sieve to a beaker and rinse the contents into the beaker with the DI water squirt bottle. Make sure all zooplankton are rinsed from the sieve by gently rinsing from behind the mesh and down the sieve wall.
5. Dilute the beaker contents to achieve ~100 to 150 organisms per mL. Do a quick count in a S-R slide if uncertain about the organism density in the beaker. Empty the S-R slide contents back into the beaker and rinse all the zooplankton off the S-R slide with a squirt bottle. Add more water if needed and record the final volume on the Zooplankton Data Sheet (Figure 5).

6. If the sample appears to be too dense for the largest beaker the lab has available (2 L), then the sample must be split using the Wildco Zooplankton splitter.
   a. Pour the sample from the beaker into the wheel portion of the splitter, using a DI water squirt bottle to rinse all the organisms from the beaker.
   b. Gently rock the wheel back and forth to mix the sample, being careful not to spill over the top opening of the wheel.
   c. Make sure buckets are in place below the wheel, then smoothly pour the sample from the wheel into the buckets.
   d. Rinse the wheel with the DI water squirt bottle and repeat steps a through c until all zooplankton have been transferred to the buckets.
   e. Pour the contents of one of the buckets into a 1 L beaker and dilute the contents to ~100 to 150 organisms per mL. If the sample still appears too dense, split the sample a second time or until the cell density is at the desired concentration.
   f. Record the split factor (S) in the notes section of the Zooplankton Data Sheet (Figure 5). The split factor is the inverse of the fraction of the original sample created by the split. A sample split one time is ½ of the original sample, therefore S = 2. If a split must be done again, then that is ¼ of the original sample, therefore S = 4.
7. Place the beaker on the magnetic stir plate. Slowly mix the sample with a magnetic stirrer at a setting between four and five to evenly distribute zooplankton throughout the beaker. Draw out a subsample with a pipette. Avoid mixing the sample at high speeds that may push larger individuals to the beaker wall. Turn off stirrer after pipetting to prevent detaching eggs from ovigerous zooplankton.
8. Place the sample onto a clean S-R slide with a cover slip. The S-R slide sample volume is 1 mL: it is important to ensure that there are no air bubbles under the cover slip after the sample has been deposited on the S-R slide as this will change the water volume and bias abundance and biomass estimates.

IDENTIFICATION

The zooplankton communities observed throughout the sampling history of Westward Region and neighboring Alaskan lakes are predominantly individuals from 2 major subclasses: Cladocerans and Copepods. Individuals within each subclass are generally keyed to genus, except the cladoceran *Daphnia* which is keyed to species when possible.

The most common cladocerans found in Alaska lakes include *Bosmina*, *Daphnia*, *Holopedium*, and *Chydorinae* (Figure 6). The most common copepods include *Cyclops*, *Diaptomus*, *Eurytemora, Epischura*, and *Harpacticus* (Figure 6). Commonly observed rotifers include *Kellicottia*, *Asplanchna*, *Keratella*, *Conochilus*, and *Filinia*, but these individuals are not counted.

All laboratory employees receive training prior to processing zooplankton samples. This training consists of a review of the laboratory's Zooplankton Identification Guide (V:\KodiakIslandLimnoLab\Zooplankton_data\Zooplankton ID Guide\ Zooplankton ID Guide 2017), a review of taxonomic references for identification (Edmondson 1959; Einsle 1996; and Sherman and Sherman 1976; Smith 1977), and a side-by-side sample test.

The following protocol provides the basic identification strategy used by the KILL. It provides a simple method for differentiating the major zooplankton sub-classes and relevant life history stages. For assistance with specific taxonomic detail, please reference the Zooplankton Identification Guide.

Identification Protocol

1. Differentiation between Cladocerans and Copepods is determined by the presence of body segments.
    a. Cladocerans are unsegmented and round with branching antennae.
    b. Copepods are segmented and elongated without branching antennae. Copepods are further distinguished as either cyclopoid or calanoid based on their body shape.
2. Ovigerous individuals are counted and measured separately.
    a. Ovigerous cladocerans store eggs in a brood pouch within the body which are visible as dark, oval structures.
    b. Ovigerous copepods have two common egg storage strategies. Ovigerous cyclopoids have one egg sack externally located on each side of their body, at the base of the body segment. Ovigerous calanoids have one egg sack centrally located towards the body segment base.
3. Individuals in early life history stages are counted but not measured.
    a. Some cladocerans have hatched from the brood pouch but are unidentifiable. These are classified as immature cladocerans.
    b. Early life stage copepods (nauplii) and cladocerans have not matured enough to allow for differentiation between taxa.

MEASUREMENTS AND ENUMERATION

A minimum of three subsamples are assessed for species composition, abundance, and size. Zooplankton lengths are collected from the first encountered individuals of each taxa. To determine the appropriate number of individuals to measure, read the subsamples and enter these data into the WIKI as described:

1. Enter the appropriate zooplankton tow metrics (e.g. depth, net diameter, dilution) into the WIKI Limnology Database through the "Add and Edit Zoo Count and Size Data" link.
    a. If the sample was split, multiply all the count results by the split factor (S) prior to entering the data into the WIKI.
    b. Document the split factor in the "Sample Comments" box in the Sample Header and save.
2. With the first subsample, identify, measure, and count the zooplankton present on the S-R slide at 200x power. *Note:* The KILL microscopes have been calibrated at the current standard magnification of 200x.
3. Record the length (mm) and number of each genus, or species where possible (i.e. *Daphnia*) on a Zooplankton Data Sheet (Figure 5). Scan each line of the S-R slide's grid following a consistent pattern.
    a. Measure the first 15 individuals of each taxa present in the subsample to the nearest 0.01 mm on the Zooplankton Data Sheet (Figure 5).
    b. Length is the distance from the top of the head to the end of the carapace on Cladocerans and from the top of the head to the end of the rami on copepods (Figure 6). Do not measure

zooplankton that are broken or oriented irregularly. Count and measure ovigerous individuals separately. Nauplii and immature cladocerans are not measured.

   c. When encountering zooplankton that cross over multiple boundaries in the S-R slide grid, be consistent with counting methods and do not count twice.

   d. For individuals that are broken, length measurements are not possible, however, they may be counted if the representative body part is singular and identifiable (e.g. heads only).

4. Discard the first subsample after reading and enter the length data into the WIKI Limnology Database to determine the number of zooplankton to measure for each taxa.

   a. The sample size required to provide a robust estimate of a population's average body length is determined using a Student's t-statistic. After measurements are saved, the WIKI calculates the student's t-statistic and provides the number to measure. This number will appear under the "Num to Measure" column under the "Calculations" tab.

5. If the number to measure has been achieved after reading the first subsample, proceed with counting taxa on the second and third subsamples as described in steps 2 and 3. If the number to measure has not been achieved, continue counting and measuring as described in steps 2 through 4 until it is met, or until 100 individuals have been measured. The minimum number of zooplankton that should be measured in each sample is 15, unless there were not 15 individuals present over the three reads.

6. Discard each subsample after counting.

7. To store a sample, re-concentrate the sample, pour back in the sample bottle, and replace the 10% neutralized formalin.

Calculations of the t-statistic, density, and biomass

1. The mean length (L; mm) and SD is calculated for the first 15 (or more) zooplankters of a genus or species, and determines the t-statistic (t) by using the formula:

$$t = \left(\frac{1.96\ x\ SD}{0.10\ x\ L}\right)^2$$

2. The number (n) of zooplankton that need to be measured by using n–1 and the t-statistic at a confidence level of 95% (Table 1) by substituting t, SD, and L into the formula:

$$n = \left(\frac{t\ x\ SD}{0.10\ x\ L}\right)^2$$

Table 1.–Student's t-statistic and sample sizes used to determine the number (n) of zooplankton to be measured to achieve a confidence level (CL) of 95% (Koenings et al. 1987).

n–1	t-statistic (95% CL)	n–1	t-statistic (95% CL)	n–1	t-statistic (95% CL)
1	12.7	11	2.2	21	2.08
2	4.3	12	2.18	22	2.07
3	3.18	13	2.16	23	2.07
4	2.78	14	2.14	24	2.06
5	2.57	15	2.13	25	2.06
6	2.45	16	2.12	26	2.06
7	2.36	17	2.11	27	2.05
8	2.31	18	2.1	28	2.05
9	2.26	19	2.09	29	2.05
10	2.23	20	2.09	> or = 30	1.96

3. Body size and biomass:
   a. Weighted mean length: uses the mean body size of a taxon combined with how numerous that taxon was on the given sample date, and gives an adjusted mean length.
   b. Biomass: uses the total mean length and a wet length to dry weight relationship specific to each taxon (Table 2) to determine biomass.
4. Zooplankton density by area: This is the total zooplankters in a sample divided by the area of the plankton net opening. Make sure the net area (A; m^2) is correct for the plankton net used for that tow (area of 0.2 m diameter net = 0.0314 m^2; area of 0.5 m diameter net = 0.1963 m^2).

$$\text{Zooplankton per m}^2 = \frac{n}{A}$$

5. Zooplankton density by volume: This is the total zooplankters (n) in a sample divided by the product of the tow depth (D; m) and the area of the plankton net opening (A; m^2).

$$\text{Zooplankton per m}^3 = \frac{n}{D\ x\ A}$$

Table 2.–Wet length to dry weight relationships for calculating zooplankton biomass. Data supplied by Jim Edmundson, personal communication.

Taxa		Biomass formulas (wet length to dry weight relationship)
Copepods		
	Cyclops	= 0.0036 * mean length $^{2.14}$ * mean density
	Diaptomus	= 0.0043 * mean length $^{2.82}$ * mean density
	Epischura	= 0.0045 * mean length $^{2.84}$ * mean density
	Eurytemora	= 0.0058 * mean length $^{1.61}$ * mean density
	Harpacticus	= 0.0036 * mean length $^{2.14}$ * mean density
Cladocerans		
	Bosmina	= 0.0102 * mean length $^{2.11}$ * mean density
	Chydorinae	= 0.0102 * mean length $^{2.11}$ * mean density
	Daphnia g.	= 0.0025 * mean length $^{3.31}$ * mean density
	Daphnia l.	= 0.0046 * mean length $^{2.17}$ * mean density
	Daphnia r.	= 0.0036 * mean length $^{2.35}$ * mean density
	Holopedium	= 0.0114 * mean length $^{2.44}$ * mean density

BASIC WATER QUALITY ANALYSIS PROCEDURES

The KILL processes lake water to derive a variety of water quality parameters. These data along with direct physical measurements and biological samples are used to inform managers about the ecosystem health of critical salmon rearing habitats. The following describes the sample analysis methods for pH, alkalinity, chl *a*, phaeo *a*, and color. The equipment and instruments used for these procedures can be found at the Manual Methods Station and in cabinet 7 (Figure 1).

PH

pH is measurement of the hydrogen ion concentration (acidity) in a solution at a specific temperature and is an important attribute in determining alkalinity (Koenings et al. 1987). pH is an important aspect of water quality and affects most chemical reactions. At the KILL, we measure pH in tandem with alkalinity on our Orion Star autotitrator.

Equipment

Orion Star T910 Series Laboratory Titrator and ROSS Ultra Electrode kit
250 mL beakers
100 mL graduated cylinder
Distilled water squirt bottle
Kimwipes

Reagents

Buffer solutions (pH 4.01, 7.00, 10.00)

Chemical Handling and Disposal

Buffer solutions are mildly hazardous; always wear gloves when handling and discard properly.

Procedure

1. Using a 100 mL graduated cylinder, measure 200 mL of the unfiltered refrigerated water sample into a 250 mL beaker.
2. Remove the bottom cap from the electrode and open the fill hole. Rinse the probe with distilled water to clean the electrode of storage solution and dry with a Kimwipe.
3. Perform a 3-point calibration of the electrode following the instructions (Figure 1; posted on CSC 27). Check the calibration summary output (Figure 1; posted on CSC 27).
4. Rinse the electrode with distilled water and blot dry with a Kimwipe.
5. Immerse the electrode in the sample, press the stir icon, and wait for the pH to stabilize.
6. Record the measurement once the green check icon appears (mV stabilized) and the number indicating pH stops blinking.
7. Rinse the electrode with distilled water and gently blot dry with a Kimwipe.
8. Continue with the next sample.
9. When finished, put the clean electrode back in the storage solution cup and cover the fill hole.

ALKALINITY

A lake's alkalinity determines its ability to resist changes in pH (Koenings et al. 1987). This buffering capacity is the product of dissolved bicarbonate salts which act to neutralize acids and bases. Chemical reactions in the water column, such as photosynthesis, can alter lake pH and alkalinity, which are important components in assessing lake ecosystem functioning. Total alkalinity (or m-alkalinity) can be determined by titration wherein all carbonate ions have been converted to carbon dioxide at a pH of 4.5.

Automated Titration

Automated titrations are performed using an Orion Star T910 Series Laboratory Titrator and the ROSS Ultra pH electrode kit. This instrument comes with a touch-screen computer that has user-friendly programmable settings and an internal QC protocol. The QC protocol stipulates that the sulfuric acid titrant must be standardized prior to each titration. The instrument standardizes the titration results according to the drift in titrant strength over time.

This method published by ThermoFisher, the Total Alkalinity Application Note (AN-ALKH2O-ERevA), is described below and is also bound with the T910 and ROSS Ultra Electrode user manuals. These documents are available both in the lab and electronically (V:\KodiakIsland LimnoLab\Meter Calibration and Manuals\Autotitrator). If the automated titrator is not functioning properly, use the manual titration method (Finkle, 2020).

Equipment

Orion Star T910 Series Laboratory Titrator and ROSS Ultra Electrode kit
1 L volumetric flask (2)
500 mL volumetric flask
100 mL graduated cylinder
Pipettes (10 mL)
Pipette tips
Distilled water squirt bottle
250 mL beakers
Kimwipes

Reagents

<u>0.01M Tris (hydroxymethyl) aminomethane (Tris/THAM)</u>: Dissolve 1.211 g of Tris (THAM) in 1 L DI water.

<u>1 N sulfuric acid (H_2SO_4)</u>: Add 27.8 mL of concentrated sulfuric acid to ~800 mL of DI water and dilute to 1 L with DI water.

<u>0.02 N sulfuric acid</u>: Dilute 10 mL of 1 N sulfuric acid to 500 mL with DI water.

<u>Buffer solutions (pH 4.01, 7.00, 10.00)</u>

Chemical Handling and Disposal

Buffer solutions are mildly hazardous; always wear gloves when handling. Concentrated sulfuric acid is very reactive and a health hazard: always wear gloves and the appropriate PPE and handle and dispose of in the fume hood. Dilute 0.02 N sulfuric acid is a mild irritant. Consult SDS.

Alkalinity Procedure

1. Prepare the electrode for use (Orion Star T910 pH Titrator; page 18). Open the fill hole before taking measurements.
2. Plug in and turn on the T910 and check the lines for bubbles. If there are bubbles in the burette or dispensing lines, click the Burette icon on the bottom left side of the screen. Select **rinse** and repeat until all bubbles are purged from the system. Do not over rinse, this wastes titrant.
3. Select **Use a Saved Method** on the home screen. Verify the titrant settings in the "KDK LK ALK" method. From the method screen select the edit (pencil) icon to the right of the method. Select **Edit Titrant**, confirm the following settings, and save:
    a. Titrant Name – 0.02 N H_2SO_4
    b. Nominal Concentration – 0.01 M
    c. Standard – THAM
    d. Standard Amount – Fixed Volume, 10 mL
    e. Standard Concentration – 0.01 M
4. Verify the titration settings for the KDK LK ALK method by selecting **Edit Titration**. The settings should be checked with each use (Figure 1; posted on CSC 27) The parameters have been selected to accommodate the KILL's low-concentration, sulfuric acid titration. Confirm the following settings and save:
    a. Titrant name and concentration – 0.02 N H_2SO_4, 0.01 M
    b. Titration Technique – Preset End Pt.
    c. Endpoint values – 4.5
    d. Sample Amount – Fixed vol, 200 mL
5. Once all settings have been verified, select the "KDK LK ALK" method. Begin calibrating the electrode by selecting **Calibrate** on the pre-titration screen. Perform the 3-buffer calibration (pH 4.01, 7, and 10) as prompted. After calibrating, rinse the probe thoroughly with distilled water and blot dry with a Kimwipe.
6. The titrant should be standardized once a month before running samples. Standardize the titrant using the following procedure:
    a. Prepare the 0.01M Tris solution. Pipet 10 mL of the 0.01 M Tris standard into three 250 mL beakers. Then pipette 110.0 mL of DI water into each beaker for a total volume of 120 mL.
    b. Select **Standardize** at the pre-titration screen and follow prompts to standardize the titrant.
    c. The new standardized titrant concentration will automatically be saved and used for subsequent KDK LK ALK method titrations.
7. Record that a calibration and standardization was performed in the alkalinity notebook. Make a table for date, lake, station, and depth of samples to be measured.

8. After calibration and standardization, select and measure 200 mL of sample, pour into an acid washed beaker and place the electrode, stirrer, ATC probe and dispenser into the sample. Make sure the reference junction on the electrode is submerged.
9. On the analysis screen select **Start Titration**. Name the sample using the convention: sample month and year, lake name (first 3–4 letters), station, and depth (e.g. 919 spir 1.50 is a sample collected in September 2019, at Spiridon Lake station 1 at 50 m).
10. When the titration is finished, record the initial pH, final concentration (alkalinity), and titrant volume added in the alkalinity notebook. Record the alkalinity and pH on the DLS form for that lake and date.
11. After each sample is complete, rinse the electrode, stirrer, ATC, and dispenser thoroughly with distilled water and blot dry with a Kimwipe. Continue following steps 8–11 until all samples have been processed.
    a. If you let the pH reading stabilize before performing the automated titration, the titration will be quicker and the results will be more accurate
12. After all samples have been measured, prepare the electrode appropriately for storage.
13. Unplug the instrument after use; power surges can damage the probe.

CHLOROPHYLL *A* AND PHAEOPHYTIN *A*

Primary production can be estimated by calculating the concentration of phytoplankton derived chlorophylls in a given water volume (Edmondson 1959). Chl *a* is the largest component of chlorophyll in the algal pigment suite. Chl *a* degrades through senescence and zooplankton grazing. Senescence creates metabolites that can interfere with the accurate determination of chl *a*. The most abundant chlorophyll degradation product is phaeo *a* (Lind 1985). The chlorophyll determination method outlined below is a modified version of Strickland and Parsons (1972) that corrects for the interfering presence of phaeo *a*. EPA method 446.0 may be followed to comply with contract requirements of lab clients. Avoid acid washing during pigment analyses.

Equipment

ThermoFisher Scientific 10s UV-Vis Spectrophotometer
10 mm glass cuvettes
Cuvette racks
Centrifuge tube racks
Acetone squirt bottle
Clay-Adams Dynac centrifuge
15 mL polypropylene centrifuge tubes and caps
Particulate samples frozen on Whatman GF/F filters
Forceps
Grinding vessel (ceramic mortar and pestle)
Volumetric flasks
Pipettes (10 L electronic, 100 µL)
Pipette tips
Lens paper
Kimwipes

Beakers (1000 mL, 500 mL, 250 mL)
Extraction box (cardboard)

Reagents

90% acetone: Dilute 900 mL of reagent-grade acetone (C_3H_6O) to 1 L with DI water.

2 N Hydrochloric acid (HCl): Add 42 mL of concentrated HCl to ~200 mL of DI water and dilute to 250 mL with DI water.

Quality control chl-*a* standard: Turner Designs (catalog # 10-850) chl-*a* standard. See manufacturer's instructions.

Chemical Handling and Disposal

Acetone use should be confined to the fume hood. Wash hands after use and avoid extended contact with fumes. HCl is a reactive health hazard, prevent contact and handle or dispose of only in the fume hood. Gloves (nitrile) should be worn at all points during this procedure. Consult SDS.

Procedure

Day 1

1. Chill and store mortars and pestles in the freezer until needed for grinding filters.
2. Prepare a chl-*a* data sheet (V:\KodiakIslandLimnoLab\KILL Experiments\Chlorophyll).
3. Place the correct number of centrifuge tubes in a rack, cap, and label with sharpie.
4. Prepare 90% acetone and pour into 500 mL squirt bottle.
5. Turn off the lights. Select a chl-*a* filter from the freezer and bring it to the fume hood. Using forceps, place the filter in the chilled mortar. Keep samples as dark as possible and refrigerated throughout the procedure.
6. Pipette 2 mL of 90% acetone into the mortar. Grind the filter with the pestle to create a slurry-like paste.
7. Pipette another 2 mL of 90% acetone into the mortar and regrind.
8. Using the pestle, scrape or pour the slurry into a 15 mL centrifuge tube. Rinse the mortar and pestle with 4 mL of 90% acetone and pour into the centrifuge tube. Repeat with 2 mL of 90% acetone. A total volume of 10 mL of acetone will be used.
9. Cap the tube immediately and place on a rack in the cardboard box in the refrigerator and keep covered and dark overnight, but no more than 22 hours, to maximize extraction.

Day 2

1. Select up to 12 samples at a time to centrifuge. Centrifuge tubes for 20 minutes at 2,500 rpm (set DYNAC centrifuge to 95). Rotate the tubes 180° and centrifuge for another 20 minutes.
2. When finished, decant the supernatant into a clean 15 mL centrifuge tube and dilute with the 90% acetone squirt bottle to a sample volume (V_s) of 12 mL. Note the supernatant volume on the datasheet before you dilute with acetone.
3. Place the extract sample in the refrigerator until ready to read on the spectrophotometer.
4. Turn on the spectrophotometer and let it warmup for 15 minutes.

5. Prepare a 90% acetone blank in a glass cuvette and place it in the spectrophotometer cuvette carousel in the spot labeled "B" for blank. Select **Set nm** and enter in the first wavelength (750 nm). Then select **Measure Blank**. It should read 0.000. Repeat this procedure to blank the spectrophotometer every time you change wavelengths.
6. Select five samples. Using a clean pipette for each sample, transfer 3 mL of sample extract from each centrifuge tube into a glass cuvette. Ensure that the outside of the cuvette is clean and dry, wiping with lens paper if needed.
7. Measure the absorbance of the chl-*a* sample against a 90% acetone blank at 750 nm and 665 nm.
8. Acidify samples with 25 µL of 2 N HCl. Wait two minutes and measure the absorbance of the acidified fraction at 750 nm, and 665 nm
9. Record the absorbances onto the data sheet. If phaeophytin measurements are negative the acid ratio is not between 1.3 and 1.7; the sample is not adequately acidified.
10. After reading all the cuvettes, remove from the carousel and empty the sample into the waste container in the fume hood. To rinse, use the squirt bottle to insert a small amount of 90% acetone into each cuvette. Cap, shake, and then pour into the waste container. Using a cotton pad gently wipe the cuvette dry and place back in the cuvette holder. The remaining acetone inside the cuvette will evaporate.

Calculations

Monochromatic method

$$\text{chl } a\ (\mu\text{g L}^{-1}) = \frac{26.7(665_O - 665_a) \times V_S}{L \times V_f}$$

$$\text{phaeo } a\ (\mu\text{g L}^{-1}) = \frac{26.7[1.7(665_O - 665_a)] \times V_S}{L \times V_f}$$

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

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

V_s = total volume (mL) of sample extract

L = path length (cm) of cuvette

V_f = volume (L) of lake water filtered

COLOR

The presence of dissolved organic compounds and colloidal particles imparts color and restricts light penetration (Wetzel and Likens, 1991), consequently impacting the depth of the euphotic zone. The platinum cobalt method uses filtered water samples.

Equipment

ThermoFisher Scientific 10s UV Vis Spectrophotometer
10 mm glass cuvettes
Cuvette rack
Pipette
Pipette tips
DI water

Reagents

500 platinum cobalt color unit standard

Chemical Handling and Disposal

The platinum cobalt standard is mildly hazardous. Wear gloves. Wash hands after use and avoid extended contact. Consult SDS for specifics.

Standards

Prepare platinum cobalt standards using the dilution matrix (Table 3). The KILL typically does not measure color concentrations exceeding 15 Pt-Co units. Produce an appropriate standard curve for each lake's estimated color range; when running new samples, consider a larger standard range (Table 3).

Procedure

1. Thaw water samples and bring to room temperature. Prepare a Color Analysis Worksheet (V:\KodiakIslandLimnoLab\Blank Data Sheets-Logs-Labels\KILL Sheets).
2. Measure the absorbance of the standards at 400 nm against a DI water blank in the spectrophotometer using 10 mm cuvettes. Record the absorbances on the Color Analysis Calculator (V:\KodiakIslandLimnoLab\KILL Experiments\Color).
3. Fit a regression to the data to calculate a linear formula and the r^2.
4. Pipette 3 mL of filtered sample water into a 10 mm cuvette directly from the sample bottle.
5. Measure the absorbance of duplicate samples at 400 nm against a DI water blank.

Calculations

Using the linear equation from the regression, calculate color in Pt-Co units.

Table 3.–Platinum cobalt standard formulas for concentrations from 0–125 Pt-Co units.

Pt-Co units	Color standard volume (mL)	DI water volume (mL)	Sample absorbance at 400 nm
0.00	0.00	4.00	0.000
12.50	0.10	3.90	0.019
25.00	0.20	3.80	0.046
37.50	0.30	3.70	0.064
62.50	0.50	3.50	0.103
125.00	1.00	3.00	0.210

AUTOMATED NUTRIENT ANALYSIS PROCEDURES

Prior to 2013, the KILL analyzed nutrients using manual chemistries (Hopkins 2017; V:\KodiakIslandLimnoLab\Methods\Manual Nutrient Chemistries). In 2012, the KILL acquired a SEAL AA3 (Figure 7) that has automated the analysis of lake nutrients (excluding TKN) since 2013. In 2018, the KILL added the capability to run TKN and can now run all nutrient chemistries on-site.

SEAL ANALYTICAL AA3 AND XY-2 SAMPLER

The SEAL AA3 with XY-2 autosampler uses the principle of air-segmented continuous flow-analysis for fully automated colorimetric analysis. Samples separated by air bubbles are mixed with reagents in a continuously flowing stream. The main components are the Sampler, Pump, Chemistry Module, and Digital Colorimeter. System control is provided by the SEAL Analytical AACE software. Due to its modular design the AA3 can be easily adapted to the specific requirements of the KILL (Figure 7). The KILL uses AA3 chemistry applications for TA, N+N, TKN, TP, TFP, FRP, and Si analyses.

AA3 COMPONENTS

XY-2 Sampler

This autosampler is used to aspirate samples and deliver them to the AA3 Chemistry Module.

AA3 Pump

The AA3 pump is a high precision peristaltic pump that moves samples, reagents, and air at defined flow rates through a series of flexible pump tubes. By a steady process of squeezing and relaxing pressure on the tubes, the fluids are drawn into the system and pushed forward through the AA3 system.

AA3 Chemistry Module

The chemical reaction takes place in the Chemistry Module. After the pump, there is a manifold containing the components required for the reaction (e.g. mixing coils, heating bath, ion exchange column, etc.). Each manifold corresponds to a single chemistry method and is mounted on the Chem Tray. Each Chemistry Module can hold two Chem Trays and determine two parameters simultaneously. At the end of the reaction, the sample/reagent mixture travels from the Chemistry Module to the Digital Colorimeter for colorimetric analysis.

AA3 High Resolution Digital Colorimeter

The High-Resolution Digital Colorimeter contains the detector module and the control electronics for all AA3 modules. The sample flows from the Chemistry Module to the colorimeter where the absorbance of the sample is read at the end of the chemical reaction.

PC with AACE Software

The AACE software is SEAL Analytical' s dedicated continuous flow software package. It controls AA3 components, stores and starts programmed runs, tracks runs, and displays reports and editing functions. For use with the AA3 HR Digital Colorimeter, the AACE software version 6.02 or higher is required. The AA3 HR4 Digital Colorimeter is supported by AACE software version 6.07 or higher.

DAILY START-UP PROCEDURE

Perform routine system maintenance and diagnostics before initiating an analysis. Ensure the AA3 performs each step in accordance with the specified guidelines before proceeding. This procedure can be found in the SEAL AA3 Operation Manual (SOM; section 6.1) and in the Customer Support Manual (CSM; section 4).

1. Switch on all AA3 modules and pump DI water and wetting agent through the reagent lines.
    a. Wetting agent is required to wet plastic tubing so that the bubbles are separated from the tubing surface by a thin film of liquid (e.g. Brij-35).
    b. Add the same type and amount of wetting agent to the DI water as specified in the method documentation for the reagent with the highest flow rate. Typical wetting agents are 15% Brij-35 and sodium dodecyl sulfate. Refer to specific nutrient test methodology for required wetting agent recipe.
2. Check bubble patterns in all lines, especially the flowcell waste line.
    a. Bubbles must completely fill the tubing. Their length should be ~1.5 times the inner diameter of the tubing.
    b. Bubble pattern must be uniform in size, shape, and distance apart.
    c. Bubble shape in all plastic tubing must be round at the front and back. If the bubble looks straight or square at the back, there is insufficient wetting agent, the tubing is the wrong type, or the tubing is contaminated.
3. Check the baseline.
    a. Start the Charting option in AACE to display charts with the channel readings.
    b. Check the baseline. It should be stable. If noise, spikes, or baseline drift is observed, correct (SOM; section 8.3).
4. Check reagent absorbance and sensitivity (SOM; section 6.3, 6.4):
    a. Before starting an Analysis for the first time.
    b. As a regular check once a week.
    c. When pump tubes are changed, or when fresh reagents are used.
5. Check bubble pattern and baseline with reagents.
    a. Place the reagent lines from the wash receptacle into the reagent containers. Check the method description if reagents must be introduced in a certain order.
    b. Check that the bubble pattern is maintained with reagents.
    c. When the reagents have reached the flowcell and the chart reading increases, check the baseline. It should still be stable and flat. If noise, spikes, or baseline drift is observed, correct (SOM; section 8.3).

DAILY SHUTDOWN PROCEDURE

At the end of the day, carry out the following shutdown procedure (SOM; section 6.2):

1. Place reagent lines into water or wash solution.
    a. Check the method description for reagent removal order and method-specific washes.

b. If the method does not specify a wash solution, use water and wetting agent (same as used for the reagents).

2. Set the pump to fast speed and pump wash solution through the complete system for 10 minutes, or until the tubing has been flushed with water and wetting agent.
3. Turn off the pump and remove the pump platen to release pressure from pump tubes.
4. Switch off all AA3 modules.

MAINTENANCE PROCEDURES

The AA3 system is regularly maintained by SEAL Analytical, who are contracted to perform annual maintenance. Daily and monthly maintenance tasks are performed by KILL lab staff and are outlined in the SOM and below. Keep maintenance records that document routine servicing events and system performance data. Record all maintenance and troubleshooting in the AA3 notebook. This also serves as a record for SEAL Analytical service engineers or for telephone troubleshooting. As part of the service agreement, technical support is also available from SEAL chemists. All written dialogues should be copied and filed in the method notebooks.

System Clean

The AA3 is run monthly while in season but requires regular cleaning for best performance. A system clean should be performed if the AA3 has been idle for more than a month, whenever pump tubes are changed, when switching between nutrient chemistries, or if there is unexplained noise in the baseline. System cleans should also be performed prior to running any of the nitrogen (N) methodologies (TA, N+N, TKN).

The system clean procedures are put in an ordered list below and have been rendered into a quick reference document (Appendix A5; Figure 1 cabinet 15).

1. Empty and acid wash all system wash flasks (Brij-35, sodium dodecyl sulfate) and DI water containers. Refill with fresh solutions or DI water and swab out the sampler wash pot with isopropyl alcohol.
2. Prepare the system cleaning solutions using 500 mL volumetric flasks:
    - 1 N HCl: 43 mL concentrated HCl, diluted to 500 mL with DI water
    - 1 N NaOH: dissolve 20 g NaOH, in 500 mL DI water
    - 10% bleach: 50 mL bleach, diluted to 500 mL with DI water
3. Turn on the AA3 and pump these cleaning solutions through the lines for 10 minutes each:
    - 1 N HCl
    - DI water
    - 1 N NaOH
    - DI water
    - 10% bleach
    - DI water
4. Return all lines to the system wash solutions (Brij-35, sodium dodecyl sulfate) or DI water bottle.

Weekly maintenance (SOM; section 7.2)

1. Once a week, or whenever reagents or pump tubes are changed, check reagent absorbance and sensitivity (SOM; sections 6.3 and 6.4).
2. Before each use, move the Pharmed tubing under the air valve to compress a new section.
3. Check the pump tubes and replace them if they are worn or dirty.

Monthly maintenance

1. When reagents or pump tubes are changed, check reagent absorbance.
2. Twice a month, turn on the AA3 (while in season) to flush the lines for easier start-up between monthly nutrient runs.
3. Perform a full system clean when changing the nutrient method.

Yearly maintenance (SOM; section 7.4; performed by SEAL technician)

1. Replace the lamp in the photometer (SOM; section 5.4.2).
2. Check the wavelength filter in the photometer to determine if it looks dark around the edge and needs to be replaced (SOM; section 5.4.1).
3. Check the small oil pads above the chain rollers in the pump and replace if worn.
4. Check the pump platen clearance (SOM; section 3.5.5).
5. Replace all transmission tubing, connections, and nipples as necessary.

For more information on maintenance intervals and procedures for the complete AA3 system, refer to the AA3 SOM.

200-hour maintenance (SOM; 7.3)

1. Change the pump tubes (Acidflex tubes every ~50–100 hours, SOM; section 3.3.1). Note that new pump tubes must run for an hour before stable readings are obtained.
2. When the tubes have been removed, take out the pump side rails and clean them with a Kimwipe moistened with isopropanol (SOM; section 3.5.2).
3. Clean the eight pump rollers (not the chains) and the bottom of the pump platen thoroughly, using a clean, lint-free cloth or paper towel moistened with isopropanol (SOM; section 3.5.1).
4. Lightly lubricate the bottom of the side rails with a thin film of Semi-Fluid Lubricant (SEAL catalog #590-0161-01).
5. Lubricate the two small oil pads situated above the chain rollers by adding two drops of all-purpose oil (SEAL catalog #538+9000-01) to each pad.
6. Lubricate the pump roller and chain bearings: put one drop of all-purpose oil (SEAL catalog # 538+9000-01) into the two oil holes between the chain rollers, near the oil pads. Place one drop of oil on each end of each pump roller and chain interface. Rotate rollers and wipe off excess oil on the pump rollers and the bottom of the pump platen (SOM; section 3.5.4).
7. Replace the air valve tubing (SOM; section 3.3.2) with Pharmed tubing (SEAL catalog # 117+0539-07) which has a longer life than silicone tubing.
8. Check reagent absorbance and sensitivity (SOM; 6.3–6.4).

AA3 System Idle

At the end of the lab's sample analysis season (December) it is important to prepare the AA3 for long term shutdown by carrying out the following procedures.

1. Remove all system wash lines from the wash bottles and run DI through the lines. After 15 minutes, either stop pumping and leave the DI water in the lines or remove the lines from the DI water and pump the water out until the system is dry.
2. Take the Pharmed air valve tubing out from under the piston press.
3. Empty, rinse, and acid wash all reagent and wash bottles. Store bottles clean.

Quality Assurance And Control Measures

QA and QC measures must be taken to ensure the KILL produces high quality data. This is especially critical when running low concentration nutrient chemistries. This necessitates attention to detail, cleanliness, and closely adhering to protocols. Please reference and comply with all measures in the general QA and QC section (page 22) as well as the following nutrient chemistry-specific protocols. The methods listed below should be documented as outlined in the database (V:\KodiakIslandLimnoLab\KILL Experiments\QC files).

STANDARDS

The KILL uses a serial dilution process to generate standards for low concentration nutrient detection (V:\KodiakIslandLimnoLab\KILL Experiments\Serial Dilution calculator.xls). This calculator uses the molecular weight of each standard compound to create high quality primary and secondary stock standards. It then calculates the amount of secondary stock standard needed to create a user-defined range of concentrations for a standard curve. When creating a standard curve, the goal is to have most of the samples fall near the center of the range (e.g. Table 4).

The recommended stock standard formulas generated from this calculator are listed below each method.

Annual Inter-Laboratory Comparison

The USGS conducts an inter-laboratory comparison study semiannually. This project provides a variety of Standard Reference Samples for laboratory quality assurance testing and internal QC. Samples are Colorado stream water spiked with reagent grade chemicals. The KILL participates annually in this inter-laboratory comparison study during the peak summer season for QC purposes.

Checking Reagent Absorbance

Check the reagent absorbance to verify that reagents meet the specifications listed in the method. The reagent absorbance should be checked every time new reagents are prepared and prior to every nutrient run (Figure 1; CSM section 4, page 8). When calculating the reagent absorbance for any given method, consult the Performance Data section in each method for the expected value. If the calculated value is outside of the specified range, remake reagents.

Certified Reference Material

Certified reference material, also known as a nutrient check standard or blind, will be run with every sample batch. The nutrient check standards currently used by the KILL (VGH Labs, Water

Pollution QC Check Sample: Simple [LGC Standards catalog # VHG-QWPSNUT-15] and Complex Nutrients [LGC Standards catalog # VHG-QWPCNUT-15]) come in 15 mL tubes with a certificate of analysis and known nutrient concentrations. The certificate of analysis provides check standard acceptable limits that reflect biases in the methods and approximate a 95% confidence interval that should be achieved through acceptable environmental methods.

Because the KILL runs low concentration nutrient chemistries, the check standards will need to be diluted to fit within the method concentration range. The serial dilution process is the most effective method for generating solutions of sufficiently low concentrations. The serial dilution calculator includes calculations for appropriate high, mid, and low concentration blind check standards which should be selected based on the nutrients and lakes being analyzed (V:\KodiakIslandLimnoLab\KILL Experiments\Serial Dilution calculator.xls).

After each check standard is analyzed the results should be documented on the data report and entered in that year's QC spreadsheet (V:\KodiakIslandLimnoLab\QC-QA\AA3\Blind and sample data logs\20XX AA3 Sample QC log.xlsx). Paper documentation from the CRM should be cataloged for reference in the Water Standards notebook stored in the lab. If the measured value is not within the advisory range: re-run. If the value remains out of range, troubleshoot by remaking standards or reagents, running a system clean, or inspecting the AA3 hardware (pump tubing, lamp, etc.).

Replicates and Redundancy

The performance data in each method description contains information on the expected SD. Run the blind check standards and at least one sample in triplicate during every nutrient analysis batch to verify that the run is performing within the provided specifications. Run method replicates to assess the quality of sample preparation steps (i.e. digestion processes used in TFP, TP, and TKN nutrient chemistries). Replicates help assess the AA3 baseline behavior.

Spikes and Blanks

Spikes and blanks are inserted into a run to ascertain sources of interference that might alter the accuracy of the run. Blank samples can help gauge how the standard curve resolves samples near the 0 intercept. Blanks are informative in identifying and correcting systematic errors due to impure reagents, contaminated glassware, and poorly calibrated instrumentation. Spiked samples are used to determine the presence of matrix effects by gauging how much standard is recovered in the presence of an unknown matrix. The standard spike preparation is 0.5 mL of secondary stock standard to 24.5 mL of a given sample. The KILL's convention for acceptable spike performance is plus or minus 10% recovery of the stock standard concentration.

Control Charting

Control charts are graphing tools used to detect when an analysis begins to drift out of statistical control. At the termination of each nutrient run, the measured concentrations from the standard curve should be plotted against the expected values. Enter the data into the control chart spreadsheet (V:\KodiakIslandLimnoLab\QC-QA\AA3\Control charting\2019 Control charts.xlsx).

Run Setup

When setting up a nutrient run, it is important to use all QC measures. Each run should contain:

1. Blanks: prepare DI water blanks in the appropriate matrix (i.e. digested).

2. Check Standards:
    a. Properly dilute blind check standards to fit the range of each nutrient chemistry.
    b. Run blind check standards in triplicate at the beginning, middle, and end of the run.
3. Spikes: run spike samples in triplicate.
4. Method replicates: always perform when running digested samples.

After each run, document the QC results on the AA3 QC Data Summary Sheet (V:\KodiakIslandLimnoLab\Blank Data Sheets-Logs-Labels\AA3 Sheets).

Run QC Criteria

For an AA3 run to be deemed satisfactory, the run must meet the following criteria:

1. Standard curve $r^2 \geq 0.999$ with a minimum of four evenly spaced standards
2. Blind check standard replicates (≥3 that lie within the sample concentration range) are within manufacturer established QC acceptance limits (typically ≤15% of certified value, but these can vary by nutrient analysis, check LGC certificate of analysis)

INTERFACING WITH AA3 SOFTWARE

Basic instructions for setting up a sample run using the AA3 software are listed below. For more detailed instructions regarding run setup, consult the Seal Analytical AACE 6.07 Software Operations Manual (MB7-60EN-07) found in the lower storage cabinet below the AA3 computer, underneath cabinet 15 (Figure 1).

Run Setup

To start a new run, go into the AA3 software menu and select **Set Up – Analysis** from the main menu. Double-click the corresponding folder for the analysis you wish to run, select the most recent run performed, and click **Copy Run**. This will save time by using the run settings and sample tray from the most recent analysis.

Main Page

When the Set Up window opens it will automatically generate a run name containing the date under the Main Page tab. To finish naming the run, type in the months the samples were collected, followed by the nutrient analysis (e.g. date of run, sample month(s), analysis; 170831JulyAugTP). If running a P analysis, under the Analysis heading, specify the method being used.

Tray Protocol Tab

The Tray Protocol tab displays the arrangement of cups on the sample tray. The tray should include a primer, a drift check, calibrants, a carryover series, blanks, check standards, spikes, replicates, and samples. To insert any of these into the tray, click the cup type buttons on the right. When selecting the low for the carryover test, make sure to use the lowest calibrant.

Channel Tab

When copying a previous run, it's important to ensure that the calibrant concentrations match those prepared according to the serial dilution calculator spreadsheet. Check the calibrant concentrations in the Channel tab. Modify accordingly.

METHODOLOGIES AND CHEMISTRY APPLICATIONS

Detailed methodologies and chemistry applications for TA, N+N, TP, TFP, FRP, TKN, and Si nutrient tests using the AA3 are listed below. For AA3 system troubleshooting, see section 8 of the SOM.

Operating Considerations

Below is a list of operational protocols to reference when preparing the AA3 for nutrient tests:

- Select drift, baseline, and carryover corrections prior to run.
- Baseline should not be included in the calibration curve.
- Do not remove the colorimeter cover during a run.
- Verify that the reagent and wash volume is adequate for the run. Refill when needed.
- Prevent waste container overflow.
- Waste lines should be short and not immersed in waste liquid to avoid back pressure.
- Check the bubble pattern before starting a run. Bubbles must be uniform in size, spacing, and round at the front and back (CSM; section 4).
- Calculate each method's sensitivity and reagent absorbance. Record this in the AA3 troubleshooting notebook and compare the results to the value in the method description.
- Never run the pump at high speed while circulating strong acid or alkali (above 2 N); this could result in a connection coming loose and spurting a corrosive reagent.

Total Ammonia (TA) in Water

For filtered water samples, measured as N:

Ranges: 0–42 µg N/L to 0–380 µg N/L and 0–0.35 µg N/L to 0–4.2 mg N/L

Method Description

This method is modified from SEAL AA3 Method no. G-171-96 Rev. 17 (Multitest MT19), specifically outlining the phenate chemistry. In this method, the Berthelot reaction occurs. This creates a blue-green colored complex measured by the colorimeter at 660 nm. A complexing agent of ethylenediamine tetra-acetic acid (EDTA) and tri-sodium citrate dihydrate prevents the precipitation of calcium and magnesium hydroxides. Sodium nitroprusside enhances the sensitivity.

Hardware: 37°C heating bath (5.4 mL) **Pump tubes:** 6 + 2 air + sampler wash

Performance Data

Range	0–140 µg N/L	0–4.2 mg N/L
Sensitivity	0.04–0.07	0.15–0.19
Reagent absorbance	0.02–0.04	0.02–0.04
Pooled SD	0.56 µg N/L	
Detection limit	0.48 µg N/L	

TA Reagents

All chemicals should be of analytical grade or equivalent and prepared with DI water. Reagent concentrations and batch volumes are diluted from the original method for low concentration analyses. The EDTA in the complexing reagent is reduced from the original method to minimize baseline noise. Use fresh reagents to avoid contamination.

Chemical List

Chemical name	chemical formula	safety classification
Ammonium sulfate	$(NH_4)_2SO_4$	–
Brij-35, 15% solution	–	–
DCI	$C_3Cl_2N_3NaO_3 \cdot 2H_2O$	oxidizing, irritant, hazard
EDTA	$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$	irritant
Hydrochloric acid, concentrated	HCl	corrosive, irritant
Phenol	C_6H_5OH	corrosive, toxic, hazard
Sodium hydroxide	NaOH	corrosive
Sodium nitroprusside	$Na_2[Fe(CN)_5NO] \cdot 2H_2O$	toxic
Tri-sodium citrate dihydrate	$C_6H_5Na_3O_7 \cdot 2H_2O$	–

Chemical Handling and Disposal

Avoid strong odors in the lab and cover all reagent containers while on-line as ammonia chemistries are highly susceptible to contamination. Always wear gloves and the appropriate PPE and handle and dispose of all waste in the designated TA waste receptacle in the fume hood. Review safety classifications and consult SDSs prior to use.

System Wash Solution	**500 mL**	**1 L**
15% Brij-35	1 mL	2 mL
DI water	to 500 mL	to 1 L

For 1 L, dilute 2 mL of 15% Brij-35 to 1 L of DI water.

Complexing Reagent	**500 mL**
EDTA	1.875 g
Tri-sodium citrate dihydrate	30 g
Sodium nitroprusside	0.125 g
DI water	to 500 mL
Brij-35	0.75 mL

For 500 mL, dissolve 1.875 g of EDTA, 30 g of tri-sodium citrate dihydrate, and 0.125 g of sodium nitroprusside in ~ 400 mL of DI water. Dilute to 500 mL with DI water. Add 0.75 mL of 15% Brij–35. Store in an amber glass bottle at room temperature. This reagent is stable for two weeks.

Dichloroisocyanuric Acid (DCI)	**100 mL**
DCI	0.5 g
DI water	to 100 mL

For 100 mL, dissolve 0.5 g of DCI in ~80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Prepare a fresh batch daily and store in glass (Appendix B1; operating notes 11 and 12).

Phenate Buffer	**100 mL**	**250 mL**
Phenol	2.5 g	6.25 g
Sodium hydroxide	1.8 g	4.5 g
DI water	to 100 mL	to 250 mL

For 100 mL, dissolve 1.8 g of sodium hydroxide and 2.5 g of phenol in ~50 mL of DI water. Dilute to 100 mL with DI water. Prepare >24 hours before analysis and store refrigerated in an amber glass bottle. This reagent is stable for one to two weeks (Appendix B1; operating note 4).

TA Standards

Primary Stock Standard	**100.02 mg N/L**
Ammonium sulfate	0.2359 g
DI water	to 500 mL

Dissolve 0.2359 g of ammonium sulfate in ~300 mL of DI water. Dilute to 500 mL with DI water and mix thoroughly. Store refrigerated in amber glass bottle for up to three months.

Secondary Stock Standard	**1.00 mg N/L**
TA Primary Stock Standard (100.02 mg N/L)	2.5 mL
DI water	to 250 mL

Dilute 2.5 mL of primary stock standard to 250 mL with DI water. Store refrigerated in an amber glass bottle for one month.

Working Standards

The serial dilution calculator spreadsheet (V:\KodiakIslandLimnoLab\KILL Experiments\Serial Dilution calculator.xls) will calculate the amount of secondary stock standard needed to create a range of concentrations for a standard curve. TA is a multitest that is run with N+N. Therefore, standards for each chemistry can be combined if running both chemistries simultaneously (Table 4). Working standards should be prepared fresh, daily.

Table 4.–Total ammonia (TA) and nitrate + nitrite (N+N) standard curve dilutions. The TA secondary stock standard is a 1.0 mg N/L ammonium sulfate solution. The N+N secondary stock standard is a 1.0 mg N/L potassium nitrate solution. Standards are prepared in 100 mL volumetric flasks.

	N+N		TA		100 mL dilution
Standard ID	Concentration (µg N/L)	2° stock (mL)	Concentration (µg N/L)	2° stock (mL)	DI water (mL)
901/908	150.00	15.00	50.01	5.00	80.00
902	100.00	10.00	30.01	3.00	87.00
903	75.00	7.50	15.00	1.50	91.00
904	30.00	3.00	5.00	0.50	96.50
905	15.00	1.50	3.00	0.30	98.20
906	5.00	0.50	1.00	0.10	99.40

TA PROCEDURE

1. Prepare the phenate buffer at least 24 hours before running samples.
2. Thaw water samples. Order samples alphabetically by lake, date, station number, and depth.
3. Prepare an AA3 Sample Order Sheet and a copy of the serial dilution calculator spreadsheet to prepare standards.
4. Make remaining reagents.
5. Prepare standards (Table 4), spikes, and blind check standards prior to each run. Prepare the check standards to the specifications provided (adjust to fit the method range).
6. Prepare a spike by choosing a random sample and adding 0.5 mL of the secondary standard to 24.5 mL of the chosen sample.
7. Bring the AA3 and XY-2 Sampler on-line. Perform a system clean.
8. Prepare the run and input sample order into the AA3 system software.
9. Place all reagents on-line and calculate reagent absorbance and sensitivity. Set the gain by manually selecting the high standard (901/908) with the XY sampler. Prepare enough high standard to sample continuously for two minutes. While setting gain, collect the TA sample waste and measure final reaction pH (Appendix B1; operating note 4). The final pH should be between 11.5 and 11.9. Adjust reagent pH as needed.
10. Pour the blind check standard, blank, spike, standards, and samples into test tubes and place in the appropriate racks. Double check sample placement with order listed in AA3 software.
11. Start the run. Monitor run to ensure the baseline and primer are found and the standard curve is acceptable. If not, abort the run and make necessary changes.

12. At run termination, check the performance of the blind check standards and replicates, and document values in the QC spreadsheet. Determine if another run is needed based on the standard curve and the performance of the QC measures.
13. If the run is satisfactory, take reagents off-line and place reagent wands back into system wash pot. Perform Daily Shutdown Procedure (page 44).

Nitrate and Nitrite (N+N) in Water

For filtered water samples, measured as N:

Ranges: 0–56 µg N/L to 0–700 µg N/L and 0–700 µg N/L to 0–10000 µg N/L

Method Description

This method is from SEAL AA3 Method no. G-172-96 Rev. 15 (Multitest MT19). A copper-cadmium column is used to reduce nitrate to nitrite, which reacts with sulfanilamide to form a diazo compound. This diazo compound then couples with N-1-naphthylethylene diamine dihydrochloride to form a purple azo dye, which is measured by the colorimeter at 550 nm.

Hardware: 37°C heating bath (5.4 mL) **Pump tubes:** 6 + 2 air + sampler wash

Performance Data

Range	0–700 µg N/L	0–10 mg N/L
Sensitivity	0.91	1.03
Reagent absorbance	0.01–0.03	0.01–0.03
Pooled SD	2.00 µg N/L	2.00 µg N/L
Detection limit	0.08 µg N/L	1.65 µg N/L

N+N Reagents

All chemicals should be of analytical grade or equivalent and prepared with DI water.

Chemical List

Chemical name	chemical formula	safety classification
Ammonium chloride	NH_4Cl	irritant
Brij-35, 15% solution	–	–
Cadmium	Cd	flammable, toxic, hazard
Copper sulfate solution, 2%	$CuSO_4$	irritant, hazard
Hydrochloric acid, concentrated	HCl	corrosive, irritant
N-1-naphthylethylenediamine dihydrochloride	$C_{12}H_{14}N_2 \cdot 2HCl$	irritant
Phosphoric acid, concentrated	H_3PO_4	corrosive
Potassium nitrate	KNO_3	oxidizing, irritant
Sodium nitrite	$NaNO_2$	oxidizing, toxic, hazard
Sulfanilamide	$C_6H_8N_2O_2S$	–

Chemical Handling and Disposal

Always wear gloves and the appropriate PPE, and handle and dispose of all waste in the designated N+N waste receptacle in the fume hood. Danger: cadmium is toxic. Avoid skin contact or inhalation of dust. Make column in the fume hood and discard all waste in the proper receptacle. Review safety classifications and consult SDS prior to use.

System Wash Solution	**500 mL**	**1 L**
Brij-35, 15% solution	3.0 mL	6.0 mL
DI water	to 500 mL	to 1 L

For 1 L, dilute 6 mL of 15% Brij-35 to 1 L with DI water.

Ammonium Chloride Reagent	**500 mL**
Ammonium chloride	5.0 g
DI water	to 500 mL
Brij-35, 15% solution	0.5 mL

Dissolve 5 g of ammonium chloride in ~ 400 mL of DI water, dilute to 500 mL. Add 0.5 mL of 15% Brij-35 and mix thoroughly. Store at room temperature in an amber glass bottle (Appendix B2; operating note 10).

Color Reagent	**100 mL**	**500 mL**
Sulfanilamide	1.0 g	5.0 g
Phosphoric acid, concentrated	10 mL	50 mL
N-1-Naphthylethylenediamine dihydrochloride	0.05 g	0.25 g
DI water	100 mL	to 500 mL

For 500 mL, add 50 mL concentrated phosphoric acid and 5 g of sulfanilamide to ~300 mL DI water. Dissolve completely. Add 0.25 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 500 mL with DI water and mix thoroughly. The reagent should be colorless: if it is pink the phosphoric acid is impure. Use a stir bar and heat if necessary for full dissolution of reagents. Store at room temperature in an amber glass bottle. This reagent is stable for a month.

Preparing the Cadmium Reductor Column

Prepare the cadmium column with the following solutions (Appendix B2; operating note 5).

2% Stock Copper Sulfate	**100 mL**
Copper sulfate	2.0 g
DI Water	to 100 mL

Dissolve 2 g of copper sulfate in ~ 60 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Alternatively, order 2% copper sulfate premade. This solution is stable indefinitely.

6 N Hydrochloric Acid	**1 L**
Hydrochloric acid, concentrated	495 mL
DI water	to 1 L

For 1 L 6 N HCl, dilute 495 mL of concentrated HCl to 1 L with DI water and mix thoroughly. Store in an amber glass bottle at room temperature. This acid is stable indefinitely.

Cadmium Column Conditioners

Nitrite Primary Conditioning Standard	**100 mg N/L**
Sodium nitrite	0.049 g
DI water	to 100 mL

Dissolve 0.049 g of sodium nitrite in ~50 mL DI water. Dilute to 100 mL and mix thoroughly. Store in an amber glass bottle at room temperature for up to three months. This solution is used to condition the cadmium column.

Nitrate Primary Conditioning Standard	**100 mg N/L**
Potassium nitrate	0.072 g
DI water	to 100 mL

Dissolve 0.072 g of potassium nitrate in ~50 mL DI water. Dilute to 100 mL and mix thoroughly. Store in an amber glass bottle at room temperature for up to three months. This solution is used to condition the cadmium column and is also the nitrate primary stock standard.

Standards

Nitrate Primary Stock Standard	**100.00 mg N/L**
Potassium nitrate	0.361 g
DI water	to 500 mL

Dissolve 0.361 g of potassium nitrate in ~ 400 mL DI water. Dilute to 500 mL and mix thoroughly. Store refrigerated in an amber glass bottle for up to three months.

Nitrate Secondary Stock Standard	**1.0 mg N/L**
Primary Nitrate Stock Standard (100 mg N/L)	2.5 mL
DI Water	to 250 mL

Dilute 2.5 mL of the nitrate primary stock standard to 250 mL with DI water. Store refrigerated in an amber glass bottle for up to one month.

Working Standards

The serial dilution calculator spreadsheet will calculate the amount of secondary stock standard needed to create a range of concentrations for a standard curve. N+N is a multitest that is run with TA. If running both chemistries simultaneously, standards are combined (Table 4). Working standards should be prepared fresh, daily.

When creating a standard curve, the goal is for most of the samples to fall near the center of the range. Table 4 (page 53) shows concentrations for a typical standard curve and can be used as a default for the TA and N + N methods. Some lakes that the KILL monitors (Buskin, Chignik, O'Malley, Spiridon, Thumb, and Uganik) routinely exhibit concentrations outside the typical range. Run these samples with a more appropriate standard curve.

PROCEDURE

1. Prepare and condition a new cadmium reductor column the day before the run (Appendix B2; operating note 5; CSM section 4, page 12). Use about 10 g of 0.3 to 0.8 mm granulation cadmium for one column.
   a. Make ammonium chloride reagent for cadmium column storage.
   b. Clean cadmium granules in a beaker with 50 mL of 6 N HCl for one minute. Decant the HCl and wash the cadmium with another 50 mL of 6 N HCl for one minute.
   c. Decant the HCl and rinse the cadmium three times with DI water.
   d. Decant the DI water and add 50 mL of 2% copper sulfate per 20 g cadmium. Swirl the cadmium until no blue color remains in solution, or when colloidal copper appears.
   e. Decant and rinse the cadmium several times with DI water until the solution runs clear.
   f. Add another 50 mL of 2% copper sulfate and wash until no blue color remains in solution, or when colloidal copper appears.
   g. Decant and rinse with DI water until the washings contain no more black colloidal copper (7–10 rinses). The cadmium granules should look silvery. From this point on, do not expose the cadmium to air. Keep it covered with water or ammonium chloride reagent.
   h. Locate the cadmium column kit in the drawer beneath the AA3. Close one end of tygon tubing (18 cm, SEAL catalog #116-0536-18) with glass wool and an N6 nipple (SEAL catalog #116-0004-01). Attach additional tubing to the end of the nipple for draining (SEAL catalog #116-0528-01).
   i. Using a ring stand and pipette tip, attach the open end of the column to the pipette tip and fill the tubing and tip with ammonium chloride reagent. Close off the drain tube at the end with yellow clamp scissors. Transfer the prepared cadmium granules to the column using a scoopula; pour them through the pipette tip and into the tubing. Minimize gaps between the cadmium particles and pack densely. Do not allow air bubbles in the column. Drain or add ammonium chloride reagent as needed with the yellow clamp scissors.
   j. Once the column is full, detach the tubing and close with glass wool and a N6 nipple. Take the free end of the drain tube and insert the nipple. Hang the column inverted overnight with the drain tubing to allow any residual air bubbles to escape.
   k. Condition the column.
      i. Run the AA3 with the ammonium chloride reagent (if you want to conserve the color reagent, leave that wand in Brij-35).
      ii. Pump 100 mg N/L nitrate conditioning standard (N+N primary stock standard) through the sample line for five minutes. Once the nitrate conditioning standard has reached the cadmium column, open the channel so that the flow is redirected through the column.
      iii. Pump 100 mg N/L nitrite conditioning standard for 10 minutes.
      iv. When the 100 mg N/L nitrite standard is washed out completely, pump the highest N+N standard (0.15 mg N/L; 901/908) through the column until the baseline is stable (~30 minutes). This procedure stabilizes the performance of the column.

l. When the reduction efficiency falls to an unacceptable level, empty the column and repack it with regenerated cadmium. Old cadmium can be re-used. Discard all cadmium waste in the N+N waste receptacle.

2. Thaw water samples. Order samples alphabetically by lake, date, station, and depth.
3. Prepare an AA3 Sample Order Sheet and copy of the serial dilution calculator spreadsheet to prepare standards.
4. Make all reagents.
5. Prepare standards (Table 4), spikes, and blind check standards prior to each run.
6. Prepare a spike by randomly choosing a sample and adding 0.5 mL of the secondary stock standard to 24.5 mL of the chosen sample.
7. Turn on AA3 and XY-2 Sampler. Perform a system clean.
8. Prepare the run in the AA3 system software. Input sample order into the AA3 system software.
9. Place all reagents on-line. After the reagents have been running for five minutes, open the 4-way valve to the cadmium column. Calculate reagent absorbance and sensitivity (set gain).
10. Pour the blind check standards, blanks, spikes, and samples into test tubes and place in the appropriate racks. Double check sample placement with order listed in AA3 software.
11. Start the run. Monitor run to ensure the primer is found and the standard curve looks good. If either fails, abort run and make necessary changes.
12. When the run is finished, close the 4-way valve to bypass the cadmium column before taking any reagents off-line.
13. At run termination, check the performance of the blind check standards and replicates, and document values in the QC spreadsheet. Determine if another run is needed based on the standard curve and performance of the QC measures.
14. If the run is satisfactory, take the reagents off-line and place the wands back in the system wash pot.
15. Perform Daily Shutdown Procedure (page 44).

Total Kjeldahl Nitrogen (TKN) in Water

For unfiltered water samples, measured as N:

Range: 0–700 µg N/L (Appendix B3; operating note 12) **and 0–2.24 mg N/L to 0–16. 8 mg N/L**

Method Description

This method is modified from USEPA Method 351.2 (Rev. 2.0 1993) and SEAL AA3 Method No. G-225-99 Rev. 9 (Multitest MT19). It is used to quantify all the organic N and TA within a water sample. Samples must undergo digestion to convert the organic N within the sample to ammonia. After digestion, in this method an emerald-green compound is formed by the reaction of ammonia, salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at a pH of 12.8–13.0. This ammonia-salicylate complex is measured by the colorimeter at 660 nm.

Hardware: 37°C heating bath (5.37 mL) **Pump tubes:** 6 + 2 air + sampler wash

Performance Data

Range	0–5.6 mg N/L
Sensitivity	0.20–0.28
Reagent absorbance	< 0.04
Pooled SD	28.01 µg N/L
Detection limit	4.20 µg N/L

TKN Reagents

All chemicals should be of Analytical Reagent grade and prepared with fresh DI water.

Chemical List

Chemical name	chemical formula	safety classification
Ammonium sulfate	$(NH_4)_2SO_4$	–
Cupric Sulfate (Copper (II) sulfate pentahydrate)	$CuSO_4 \cdot 5H_2O$	toxic, harmful
DCI	$C_3Cl_2N_3NaO_3 \cdot 2H_2O$	oxidizing, irritant, hazard
EDTA	$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$	irritant
Potassium Sulfate	K_2SO_4	–
Sodium hydroxide	NaOH	corrosive
Sodium hydrogen phosphate-12-hydrate	$Na_2HPO_4 \cdot 12H_2O$	–
Sodium nitroprusside	$Na_2[Fe(CN)_5NO] \cdot 2H_2O$	toxic
Sodium salicylate	$C_7H_5NaO_3$	irritant
Sulfuric acid, concentrated	H_2SO_4	corrosive
Tri-sodium citrate dihydrate	$C_6H_5Na_3O_7 \cdot 2H_2O$	–

Chemical Handling and Disposal

Avoid strong odors in the lab and cover all reagent containers while on-line as ammonia chemistries are highly susceptible to contamination. Always wear gloves and the appropriate PPE, and handle and dispose of all waste in the designated TKN waste receptacle in the fume hood. Review safety classifications and consult SDS prior to use.

Digestion Reagent	**500 mL**	**1 L**
	100 samples	*200 samples*
Potassium Sulfate	67 g	134 g
Sulfuric acid, concentrated	67 mL	134 mL
Cupric Sulfate (Copper (II) sulfate pentahydrate)	5.7 g	11.4 g
DI water	to 500 mL	to 1 L

For 500 mL, add 67 g potassium sulfate to ~300 mL DI water. Carefully add 67 mL of ultra-pure, double-distilled concentrated sulfuric acid. Caution, the flask will become warm. Add 5.7 g cupric sulfate. Add water as needed and stir to dissolve. Cool. Dilute to 500 mL with DI water and invert to mix. Store in an amber glass bottle at room temperature. This reagent is stable for 1 month.

Sampler Wash Solution	**500 mL**	**1 L**
Sulfuric acid, concentrated	20 mL	40 mL
Cupric Sulfate	0.57 g	1.14 g
DI water	to 500 mL	to 1 L

For 1 L, add 40 mL of sulfuric acid to ~500 mL DI water. Add 1.14 g cupric sulfate. Mix thoroughly and dilute to 1 L. The addition of cupric sulfate allows the baseline to match the digests (Mallory Mueller, Chemist, SEAL Analytical, Kodiak, personal communication). Store in an amber glass bottle at room temperature. This solution is stable for one month (Appendix B3; operating note 1).

System Wash Solution	**500 mL**	**1 L**
Brij-35, 15% solution	3.0 mL	6.0 mL
DI water	to 500 mL	to 1000 mL

For 1 L, dilute 6 mL of 15% Brij-35 to 1 L of DI water.

TKN Buffer	**500 mL**
EDTA	9.205 g
Sodium hydroxide (~30.5 g/L):	
Range 0–700 µg N/L	18.00 g
Sodium hydrogen phosphate-12-hydrate	20 g
DI water	to 500 mL

Dissolve 9.205 g of EDTA and 15.25 g of sodium hydroxide in ~ 400 mL of DI water. Add 20 g of sodium hydrogen phosphate-12-hydrate and dilute to 500 mL with DI water. Mix thoroughly. Store at room temperature in an amber glass bottle. This buffer is stable for as long as the solution remains clear, usually one week. (Appendix B3; operating notes 5 and 12).

Sodium Salicylate	**200 mL**	**500 mL**
Sodium salicylate	63.2 g	158 g
Sodium nitroprusside	0.16 g	0.4 g
DI water	to 250 mL	to 500 mL

For 200 mL, dissolve 63.2 g of sodium salicylate and 0.16 g of sodium nitroprusside in ~150 mL of DI water. Dilute to 200 mL and mix thoroughly. Store at room temperature in an amber glass bottle. This solution is table for one week.

Dichloroisocyanuric Acid (DCI)	**100 mL**
DCI	0.45 g
DI water	to 100 mL

Dissolve 0.45 g of DCI in ~80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Make fresh daily.

TKN STANDARDS

Primary Stock Standard	**100.01 mg N/L**
Ammonium sulfate	0.2359 g
DI water	to 500 mL

Dissolve 0.2359 g of ammonium sulfate in ~ 400 mL of DI water. Dilute to 500 mL with DI water and mix thoroughly. Store refrigerated in an amber glass bottle for up to three months.

Secondary Stock Standard	**10 mg N/L**
TKN primary stock (100.01mg N/L)	25 mL
DI water	to 250 mL

Dilute 25 mL of TKN primary stock standard in a 250 mL volumetric flask with DI water.

Working Standards (Appendix B3; operating notes 1 and 2)

Calculate working standards using the serial dilution calculator spreadsheet. Calculate the amount of secondary stock standard needed to create a range of concentrations for a standard curve.

Table 5.–Total Kjeldahl nitrogen (TKN) standard curve dilutions. Standards are prepared in 25 mL volumetric flasks.

	TKN		25 mL dilution
Standard	Concentration (µg N/L)	2° stock (mL)	DI water (mL)
901/908	700.06	1.75	23.25
902	500.04	1.25	23.75
903	250.02	0.62	24.38
904	100.01	0.25	24.75
905	50.00	0.12	24.88
906	24.80	0.06	24.94

TKN PROCEDURE

1. Make digestion reagent.
2. Prepare standards (Table 5), spike, and blind check standards. Prepare blinds to the specifications provided (adjusting the dilution factor to fit the range of the method).
3. Prepare a spike by choosing a random sample and adding 0.5 mL of the secondary stock standard to 24.5 mL of the chosen sample.
4. Prepare an AA3 Sample Order sheet and a copy of the serial dilution calculator spreadsheet to prepare standards.
5. Acid wash all TKN digestion tubes, digestion boiling chips, stoppers, and AA3 test tubes.
6. Pipet 25 mL of sample, standard, blind check standard, or blank in each digestion tube.
7. Pipet 5 mL of digestion reagent into each digestion tube and vortex.
8. Add four to five Teflon boiling chips. **Caution:** An absence or excess of Teflon chips may cause the sample to splatter or boil over.
    a. To reduce the occurrence of splatter do not pre-heat the block. To maximize digestion recovery, use the SEAL Analytical BD-50 block controller to step through the three temperatures of Kjeldahl digestion.
    b. Program the block controller to the following settings:

(1) Step to 160°C	(2) Hold [30 min]
(3) Ramp to 210°C at 5°C per min	(4) Hold [30 min]
(5) Ramp to 380°C at 5°C per min	(6) Hold [40 min]

    c. During step 5, place the glass TKN stoppers in each digestion tube to minimize splatter.
    d. When digestion is complete, lift the rack of digestion tubes above the block to cool. After 10 minutes, begin dispensing 25 mL of freshly made DI water in each tube before the sample residue solidifies. Stir with a vortex mixer. Mixing will stir up sediment. Allow the sediment to settle prior to analysis (at least two hours).
9. Make the remaining reagents.
10. Bring the AA3 and XY-2 Sampler on-line. Perform a system clean.
11. Prepare the run in the AA3 system software. Input sample order into AA3 system software.
12. Place all reagents on-line except salicylate (Appendix B3; operating note 6).
13. Flush the sampler wash receptacle with 25 mL of 4% sulfuric acid.
14. When reagents have been pumped completely through the system (~7 minutes), place the salicylate wand in the reagent container and allow the system to equilibrate. If a precipitate forms after adding salicylate, the pH is too low. Stop the pump and flush the line with water. Before restarting the system, check the concentration of the sulfuric acid and buffer solutions.
15. Calculate reagent absorbance and sensitivity and set the gain using the highest standard.
16. While setting the gain, collect the sample waste and measure the final pH (Appendix B3; operating note 5). It should be between 12.8 and 13.0. Adjust reagent pH as necessary.

17. After a stable baseline has been obtained, pour the blind check standards, blank, standards, and samples into test tubes and place in the appropriate racks. Double check sample placement with order listed in AA3 software.
18. Start the run. Monitor run to ensure the baseline and primer are found and the standard curve is acceptable. If not, abort run and make necessary changes.
19. At run termination check the performance of the blank, blind check standards, spike, and replicates, and document values in the QC spreadsheet. Determine if another run is needed based on the performance of the QC measures.
20. If the run is satisfactory, take the reagents off-line, removing the salicylate first and allowing it to clear the line before removing the other reagents. Perform Daily Shutdown Procedure (page 44).

Phosphate in Water

For unfiltered water samples (TFP, FRP) and filtered water samples (FRP), measured as P

Range: 0–50 µg P/L to 0–500 µg P/L

Method Description

This method is modified from SEAL AA3 Method-No. G-297-03 Rev. 3 (Multitest MT 19). The automated procedure for the determination of orthophosphate is based on the colorimetric method in which the reaction of orthophosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH <1 (Murphy and Riley, 1962) forms a blue color. The reduced blue phospho-molybdenum complex is read on the colorimeter at 880 nm. TP and TFP samples and standards undergo a persulfate digestion prior to analysis that converts organic forms of P to orthophosphate.

Hardware: 37°C heating bath (5.37 mL)   **Pump tubes:** 6 + 2 air + sampler wash

Performance Data

Range	0–50 µg P/L
Sensitivity	0.016–0.020
Reagent absorbance	0.009–0.011
Pooled SD	0.18 µg P/L
Detection limit	0.29 µg P/L

P Reagents

All chemicals should be of Analytical Reagent grade or equivalent and prepared with fresh DI water.

Chemical List

Chemical name	chemical formula	safety classification
Acetone	C_3H_6O	flammable, irritant, toxic
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}$	irritant
Antimony potassium tartrate	$C_8H_4K2O_{12}Sb_2 \cdot 3H_2O$	irritant, hazard
Ascorbic acid	$C_6H_8O_6$	–
Potassium dihydrogen phosphate	KH_2PO_4	–
Potassium persulfate	$K_2S_2O_8$	oxidizing, irritant, hazard
Sodium dodecyl sulfate	$NaC_{12}H_{25}SO_4$	flammable, corrosive, toxic
Sulfuric acid, concentrated	H_2SO_4	corrosive, irritant

Chemical Handling and Disposal

Always wear gloves and the appropriate PPE and handle and dispose of all waste in the designated P waste receptacle in the fume hood. Review safety classifications and consult SDS prior to use.

System Wash Solution	**500 mL**	**1 L**
Sodium dodecyl sulfate	4.0 g	8.0 g
DI water	to 500 mL	to 1000 mL

For 1 L, dissolve 8 g of sodium dodecyl sulfate in ~800 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly. Use ultra-pure sodium dodecyl sulfate. Store in a polyethylene bottle at room temperature.

Stock Antimony Potassium Tartrate	**100 mL**	**250 mL**
Antimony potassium tartrate	2.3 g	5.75 g
DI water	to 100 mL	to 250 mL

For 100 mL, dissolve 2.3 g of antimony potassium tartrate in ~80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Store refrigerated in an amber glass bottle. This solution is stable for one month.

Ammonium Molybdate Reagent	**250 mL**	**500 mL**
Ammonium molybdate	0.75 g	1.5 g
Sulfuric acid, concentrated	8 mL	16.0 mL
Stock antimony potassium tartrate	2.75 mL	5.5 mL
DI water	to 250 mL	to 500 mL

For 500 mL, add 16 mL of concentrated sulfuric acid to ~300 mL of DI water and cool. Dissolve 1.5 g of ammonium molybdate and add 5.5 mL of stock antimony potassium tartrate. Dilute to 500 mL with DI water and mix thoroughly. Store in an amber glass bottle at room temperature. This solution is stable for one month and must be colorless.

Ascorbic Acid	**250 mL**	**500 mL**
Ascorbic acid	0.5 g	1.0 g
Acetone	2.5 mL	5 mL
Sodium dodecyl sulfate	0.45 g	0.9 g
DI water	to 250 mL	to 500 mL

For 250 mL, dissolve 0.5 g of ascorbic acid in ~100 mL of DI water. Add 2.5 mL of acetone and 0.45 g of sodium dodecyl sulfate. Dilute to 250 mL with DI water and mix thoroughly. Store refrigerated in a polyethylene bottle. This solution is stable for one week. Acetone is used as a preservative; do not add if making ascorbic acid fresh each run.

Digestion Reagent

	500 mL	1 L
	100 samples	*200 samples*
Sulfuric acid, concentrated	5 mL	10 mL
Potassium persulfate	30 g	60 g
DI water	to 500 mL	to 1000 mL

For 100 samples or less, fill a 500 mL volumetric flask with about 250 mL water. Add 5 mL 3.6 N sulfuric acid and dissolve 30 g potassium persulfate (Appendix B4; operating note 6). Dilute to 500 mL with DI water. Store in an amber glass bottle. Stable indefinitely.

P Standards

Primary Stock Standard **100.01 mg P/L**

Potassium dihydrogen phosphate, H_2KPO_4	0.2197 g
DI water	to 500 mL

Dissolve 0.2197 g of potassium dihydrogen phosphate in ~300 mL of DI water. Dilute to 500 mL with DI water and mix thoroughly. Refrigerate in an amber glass bottle for up to three months.

Secondary Stock Standard **1.00 mg P/L**

Primary Stock Standard (100.01 mg P/L)	2.5 mL
DI water	to 250 mL

Dilute 2.5 mL of Primary standard to 250 mL with DI water. Store in an amber glass bottle for up to one month. Prepare working standards as required.

Working Standards

Working standards can be calculated using the serial dilution calculator spreadsheet. This will calculate the amount of secondary stock standard needed to create a range of concentrations for a standard curve. Samples and standards for both TP and TFP are digested prior to analysis and are prepared in a smaller volume (25 mL) than undigested FRP standards (100 mL). Make sure to account for the difference in volume when calculating standard concentrations. The table below is provided as an example and can be used as a default for these methods. Working standards should be prepared fresh, daily.

Table 6.–Phosphorus (P) standard curve dilutions. The concentration of the secondary stock standard is 1.00 mg P/L. Total P (TP) and total filterable P (TFP) standards are digested prior to analysis and are prepared in smaller volumes (25 mL) than undigested filtered reactive P (FRP) standards (100 mL).

		FRP	100 mL dilution	TP / TFP	25 mL dilution
P standard ID	Concentration (µg P/L)	2° standard volume (mL)	DI water volume (mL)	2° standard volume (mL)	DI water volume (mL)
901/908	30.00	3.00	97.00	0.75	24.25
902	20.00	2.00	98.00	0.50	24.50
903	10.0	1.00	99.00	0.25	24.75
904	5.00	0.50	99.50	0.12	24.88
905	2.00	0.20	99.80	0.05	24.95
906	1.00	0.10	99.90	0.02	24.98

P PROCEDURE

1. Thaw water samples. Order samples alphabetically by lake, date, station number, and depth.
2. Perform digestion if running TP or TFP. If running FRP, skip to step 3.
    a. Prepare an AA3 Sample Order sheet.
    b. Bring all water samples, standards, blind check standards, and spikes to room temperature. Use unfiltered water samples to quantify TP and filtered water samples to quantify TFP.
    c. Follow the AA3 Sample Order Sheet closely using pre-numbered acid washed 40 mL glass scintillation vials for water samples, blanks, blinds, and standards (Table 6, above).
    d. Pipette 25 mL of sample, blind check standard, blank, or standard into a 40 mL glass scintillation vial.
    e. Pipette 5 mL of digestion reagent into each vial, cap, and vortex. Loosen cap so that it can vent steam during the digestion.
    f. Place samples on an autoclave tray, insert into the Tuttnauer 2540E-B/L autoclave, and seal the circular door.
    g. Fill water reservoir with distilled water. If the water level is too low, an alarm will sound.
    h. Toggle the green power switch to on and select program 3 for liquids (indicated by button with flask pictogram). The program settings are as follows:
        i. Sterilization ramp to 121°C (250°F)
        ii. Sterilization time: 30 minutes
        iii. Slow exhaust to 85°C (185°F): 15–20 minutes
    i. When the timer sounds, turn power off and slowly crack open the autoclave door using the black circular handle to allow steam to vent. Keep extremities clear of opening before venting and wear heat safe gloves when handling sample tray.
    j. Restart autoclave process with additional samples if needed.
    k. Allow samples to cool on the lab counter.

l. Once cooled, close scintillation vials and invert to mix. Place digested samples in the refrigerator. Digested samples and standards are stable indefinitely.

3. Prepare an AA3 Sample Order sheet and input run order into AA3 system software and a copy of the serial dilution calculator spread sheet to prepare the standards. Input sample order into AA3 system software.
4. Prepare standards (Table 6), spike, and blind check standards just prior to each run. Prepare the blinds to the specifications provided (adjusting the dilution factor to fit the range of the method).
5. Prepare a spike by choosing a sample (haphazard selection) and adding 0.5 mL of the secondary stock standard to 24.5 mL of the chosen sample.
6. Make remaining reagents.
7. Bring the AA3 and XY-2 Sampler on-line. Perform a system clean.
8. Place all reagents on-line and calculate reagent absorbance and sensitivity (set gain).
9. Pour the blind check standards, blanks, standards, and samples into test tubes and place in test tube racks. Double check sample placement with order listed in AA3 software.
10. Start the run. Monitor run to ensure the baseline and primer are found and the standard curve is acceptable. If not, abort run and make necessary changes.
11. At run termination, check the performance of the blind check standards and replicates, and document values in the QC spreadsheet. Determine if another run is needed based on the standard curve and the performance of the QC measures.
12. If the run is satisfactory, take reagents off-line and place back into system wash pot. Perform Daily Shutdown Procedure (page 44).

Reactive Silicon in Water

For unfiltered water samples, measured as Si:

Ranges: 0–234 µg Si/L to 0–2.8 mg Si/L and 0–3.27 mg Si/L to 0–28.02 mg Si/L

Method Description

This method is modified from SEAL AA3 Method no. G-177-96 Rev. 10 (Multitest MT 19). It is based on the reduction by ascorbic acid of silico-molybdate in acidic solution to molybdenum blue. Oxalic acid minimizes interference from phosphates. Read by the colorimeter at a wavelength of 820 nm.

Hardware: 37°C heating bath (5.37 mL) **Pump tubes:** 6 + 2 air + sampler wash

Performance Data

Range	**0–1.2 mg Si/L**	**0–28.0 mg Si/L**
Sensitivity	0.36–0.44	0.75–0.92
Reagent absorbance	0.01–0.03	0.01–0.03
Pooled SD	1.40 µg Si/L	
Detection limit	0.45–0.84 µg Si/L	

Reagents

All chemicals should be of Analytical Reagent grade or equivalent and made with DI water. The use of plastic labware is recommended to avoid silica contamination from glass.

Chemical List

Chemical name	chemical formula	safety classification
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}$	irritant
Ascorbic acid	$C_6H_8O_6$	–
Oxalic acid	$C_2H_2O_4$	corrosive, irritant
Sodium dodecyl sulfate	$NaC_{12}H_{25}SO_4$	flammable, corrosive, toxic
Sodium metasilicate nonahydrate	$H_{18}Na_2O_{12}Si$	corrosive, irritant
Sulfuric acid, concentrated	H_2SO_4	corrosive

Chemical Handling and Disposal

Always wear gloves and the appropriate PPE, and handle and dispose of all waste in the designated Si waste receptacle in the fume hood. Review safety classifications and consult SDS prior to use.

System Wash Solution	**500 mL**	**1L**
Sodium dodecyl sulfate	1.0 g	2.0 g
DI water	to 500 mL	to 1000 mL

For 1 L, dissolve 2 g of sodium dodecyl sulfate in ~800 mL of DI water. Dilute to 1 L with DI water and mix thoroughly. Use ultra-pure sodium dodecyl sulfate. Store in a polyethylene bottle at room temperature.

Ascorbic Acid	**250 mL**	**500 mL**
Ascorbic acid	12.5 g	25 g
DI water	to 250 mL	to 500 mL

For 500 mL, dissolve 25 g of ascorbic acid in ~300 mL of DI water. Dilute to 500 mL with DI water and mix thoroughly. Store in a polyethylene container at room temperature. This solution is stable for one week.

Ammonium Molybdate	**250 mL**	**500 mL**
Ammonium molybdate	3.75 g	7.5 g
Sulfuric acid, concentrated	1.05 mL	2.1 mL
Sodium dodecyl sulfate	1.25 g	2.5 g
DI water	to 250 mL	to 500 mL

For 500 mL, dissolve 7.5 g of ammonium molybdate in ~300 mL of DI water. Add 2.1 mL of concentrated sulfuric acid. Add 2.5 g of sodium dodecyl sulfate and mix. Dilute to 500 mL with DI water and mix thoroughly. The solution should be clear and free of precipitate upon standing. If blue in color, discard the solution. Store in an amber polyethylene container. This solution is stable for two weeks.

Oxalic Acid	**250 mL**	**500 mL**
Oxalic acid	23.75 g	47.5 g
DI water	to 250 mL	to 500 mL

For 500 mL, dissolve 47.5 g of oxalic acid in ~ 400 mL of DI water and mix thoroughly. Dilute to 500 mL with DI water. Store in an amber polyethylene container for up to three days. Do not refrigerate (operating note 13).

Si STANDARDS

Primary Stock Standard	**100.01 mg Si/L**
Sodium metasilicate nonahydrate	0.506 g
DI water	to 500 mL

Dissolve 0.506 g of sodium metasilicate nonahydrate in ~200 mL of DI water. Dilute to 500 mL with DI water. Do not store in glass. Stock standard is good for three months.

Secondary Stock Standard	**10.00 mg Si/L**
Primary Stock Standard (100.01 mg Si/L)	25 mL
DI water	to 250 mL

Dilute 25 mL of Primary standard to 250 mL with DI water. Do not store in glass. Stock standard is good for three months.

Table 7.–Silicon (Si) standard curve dilutions. The concentration of the secondary stock standard is a 10.00 mg Si/L sodium metasilicate solution.

	Si			100 mL dilution	SiO_2
Si standard ID	Concentration (µg Si/L)	Concentration (mg Si/L)	2° standard (mL)	DI water (mL)	Concentration (mg SiO_2/L)
901/908	4000.36	4.00	40.00	60.00	8.57
902	3000.27	3.00	30.00	70.00	6.42
903	2000.18	2.00	20.00	80.00	4.28
904	1000.09	1.00	10.00	90.00	2.14
905	500.04	0.50	5.00	95.00	1.07
906	100.01	0.10	1.00	99.00	0.21

Si PROCEDURE

1. Thaw water samples. Order samples alphabetically by lake, date, station number, and depth.
2. Prepare an AA3 Sample Order Sheet and a copy of the serial dilution calculator spreadsheet to prepare standards. Input sample order into AA3 system software.
3. Prepare standards (Table 7) and blind just prior to each run. Prepare the blind (nutrient check standard) to the specifications provided (adjusting the dilution factor to fit the range of the method).
4. Make all reagents.
5. Bring the AA3 and XY-2 Sampler on-line. Perform a system clean if the machine was last used for another nutrient chemistry.
6. Place all reagents on-line and calculate reagent absorbance and sensitivity (set gain).
7. Pour the blind check standards, blanks, standards, and samples into test tubes and place in the appropriate racks. Double check sample placement with order listed in AA3 software.
8. Start the run. Monitor run to ensure the baseline and primer are found and the standard curve is acceptable. If not, abort run and make necessary changes.
9. At run termination, check the performance of the check standards and replicates, and document values in the QC spreadsheet. Determine if another run is needed based on the performance of the QC measures.
10. If the run is satisfactory, take reagents off-line and place back into system wash pot. Perform Daily Shutdown Procedure (page 44).

ACKNOWLEDGEMENTS

Thanks are extended to Kodiak Regional Aquaculture Association, Cook Inlet Aquaculture Association, The Sitka Tribe of Alaska, Norton Sound Economic Development Corporation, and the National Park Service for supporting the monitoring and analytical efforts of the lab. Multiple projects from ADF&G regions I, II, and III have also supported the lab's operation. References to trade names do not imply endorsement by the Alaska Department of Fish and Game. Thank you to Heather Finkle, Marina Thomas, and Annie Brewster for providing comments and suggestions for improving this document.

REFERENCES CITED

Eaton, A., editor. 1998. Standard methods for the examination of water and wastewater, 20th edition. American Water Works Association, Washington D.C.

Edmondson, W. T., editor. 1959. Freshwater biology, second edition. John Wiley and Sons, Inc. USA.

Einsle, U. 1996. Guides to the identification of the microinvertebrates of the continental waters of the world volume 1: Copepoda: Cyclopoida: genera Cyclops, Magacyclops, Acanthocyclops. SPB Academic Publishing, Amsterdam, Netherlands.

Finkle, H. 2020. Kodiak Island Limnology Laboratory analysis operational plan, 2020–2022. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Operational Plan No. ROP.CF.4K.2020.09, Kodiak.

Grasshoff, P. Methods of seawater analysis. Verlag Chemie, 2nd edition. 1983.

Hopkins, A. M. 2017. Kodiak Island Limnology Laboratory analysis operational plan, 2017–2019. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report No. 4K19-05, Kodiak.

Koenings, J. P., J. A. Edmundson, G. B. Kyle, and J. M. Edmundson. 1987. Limnology field and laboratory manual: methods for assessing aquatic production. Alaska Department of Fish and Game, FRED Division Report Series 71, Juneau.

Lind, O. T. 1985. Handbook of common methods in limnology. Kendall/Hunt publishing company. USA.

Mauger, S., R. Shaftel, E. J. Trammell, M. Geist, and D. Bogan. 2014. Stream temperature data collection standards and protocols for Alaska: Minimum standards to generate data useful for regional-scale analyses. Cook Inletkeeper, Homer, AK and Alaska Natural Heritage Program, University of Alaska, Anchorage.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27:31–36.

Pyle, B., H. Finkle, T. Dodson, and T. Lance. 2014. Strategic plan for voluntary, network-based water temperature monitoring of salmon habitat in the Kodiak Archipelago, Alaska. Report prepared for the Western Alaska Landscape Conservation Cooperative. U.S. Fish and Wildlife Service, Kodiak National Wildlife Refuge, Kodiak.

Schrof, S. T., S. G. Honnold, C. J. Hicks, and J. A. Wadle. 2000. A summary of salmon enhancement, rehabilitation, evaluation, and monitoring efforts conducted in the Kodiak management area through 1998. Alaska Department of Fish and Game, Division of Commercial Fisheries, Regional Information Report No. 4K00-57, Kodiak.

Sherman, I. W., and V. G. Sherman. 1976. The invertebrates: function and form a laboratory guide, second edition. Macmillan Publishing Co., Inc. USA.

Smith, D. L. 1977. A guide to marine coastal plankton and marine invertebrate larvae. Kendall/Hunt publishing company. USA.

Strickland, J. D. H., and R. R. Parsons. 1972. A practical handbook of seawater analysis. Fishery and Research Board of Canada, Supply and Services Canada. Ottawa, Canada.

Thomsen, S. E. 2008. Kodiak Island lake assessment/limnology project and laboratory analyses operational plan. Alaska Department of Fish and Game, Commercial Fisheries Division, Regional Information Report No. 4K08-4, Kodiak.

Weaver, S. A. 2022. Westward Region lake sampling and Kodiak Island Limnology Laboratory processing schedule, 2022. Alaska Department of Fish and Game, Regional Operational Plan No. ROP.CF.4K.2022.04, Kodiak.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analyses, second edition. Springer-Verlag, New York.

FIGURES

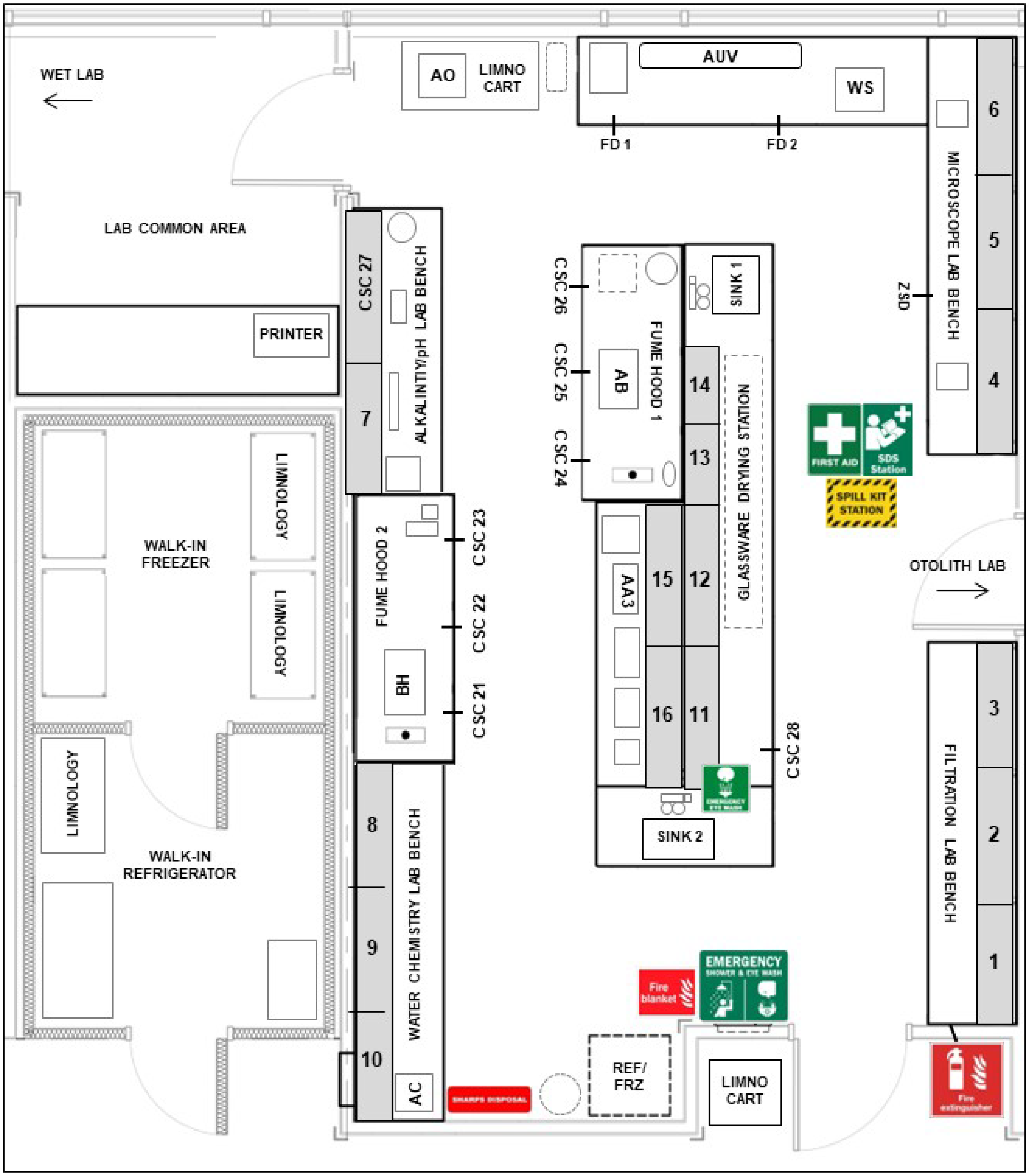

Figure 1.–Kodiak Island Limnology Lab (KILL) equipment and safety layout. The KILL water chemistry lab has two fume hoods, two sinks, a water chemistry analysis lab bench, an alkalinity/pH lab bench, a microscope lab bench with two microscopes, a filtration lab bench, seven chemical storage cabinets (CSC), two carts, an autoclave (AC), an acid bath (AB), two file drawers (FD), a glassware drying station, a block heater (BH), an automated underwater vehicle (AUV), an annealing oven (AO), a refrigerator/freezer (REF/FRZ), a computer workstation (WS), a SEAL AutoAnalyzer 3 (AA3), a zooplankton sample drawer (ZSD), and multi-use cabinets (1–15). The KILL lab is located next to the walk-in refrigerator and freezer which have designated limnology shelves for sample storage.

Nutrient chemistry	Reagent	Shelf life	Storage bottle	Storage temperature
Phosphorus	Ammonium molybdate reagent	Indefinitely	PE	Refrigerator
FRP	Ascorbic acid	1 month	PE	Refrigerator
TFP	Digestion reagent	Indefinitely	PE	Refrigerator
TP	Stock antimony potassium tartrate	1 month	Amber glass	Refrigerator
Nitrogen	Complexing reagent	Indefinitely	Amber glass	Room
TA	Dichloroisocyanuric acid (DCI)	1 day	Glass	Refrigerator
	Phenate buffer	>24 hours, 2 weeks	Amber glass	Refrigerator
N + N	Ammonium chloride reagent	1 month	Amber glass	Room
	Color reagent	1 week	Amber glass	Room
TKN	Buffer	1 week	Amber glass	Room
	DCI	1 day	Glass	Refrigerator
	Digestion reagent	1 month	Amber glass	Room
	Sodium salicylate	1 day	Amber glass	Room
Silicon	Ascorbic acid	1 week	PE	Room
	Ammonium molybdate	2 weeks	PE	Room
	Oxalic acid	Indefinitely	PE	Room

Figure 2.–Reagent shelf lives. Reagents may need to be stored refrigerated or at room temperature in glass, amber glass, or polyethylene (PE) bottles. Phosphorus chemistries include filtered reactive phosphorus (FRP), total filterable phosphorus (TFP), and total phosphorus (TP). Nitrogen chemistries include total Kjeldahl nitrogen (TKN), nitrate and nitrite (N+N), and total ammonia (TA).

Detailed Lake Survey Field Form - ADF&G Commercial Fisheries, Limnology

Lake: Station: Bottom depth (m):

Weather & lake surface conditions:

Date: Time: Sampling crew:

Physical Parameters

Depth (m)	Temperature (°C)	Dissolved Oxygen (mg/L)	Depth (m)	Photosynthetically Active Radiation (μmol m^{-2} s^{-1})
surface			surface	
0.5			0.5	
1			1	
1.5			1.5	
2			2	
2.5			2.5	
3			3	
3.5			3.5	
4			4	
4.5			4.5	
5			5	
6			6	
7			7	
8			8	
9			9	
10			10	
11			11	
12			12	
13			13	
14			14	
15			15	
16			16	
17			17	
18			18	
19			19	
20			20	
21			21	
22			22	
23			23	
24			24	
25			25	
30			26	
35			27	
40			28	
45			29	
50			30	

Isotopes Collected? yes / no

Water samples:

Samples collected? yes / no

Sampled depths (m):

1		29	
5		30	
10		35	
15		40	
20		45	
25		50	

Zooplankton:

Tow depth: __________ (m)

Secchi disk:

Disappeared: __________ (m)

Reappeared: __________ (m)

Mean disk reading: __________ (m)

pH & alkalinity:

field - 1m pH: ______

lab - 1m pH: ______ alk: ______**mg/L $CaCO_3$**

___m pH: ______ alk: ______**mg/L $CaCO_3$**

Field notes:

Figure 3.–Detailed Lake Survey (DLS) form.

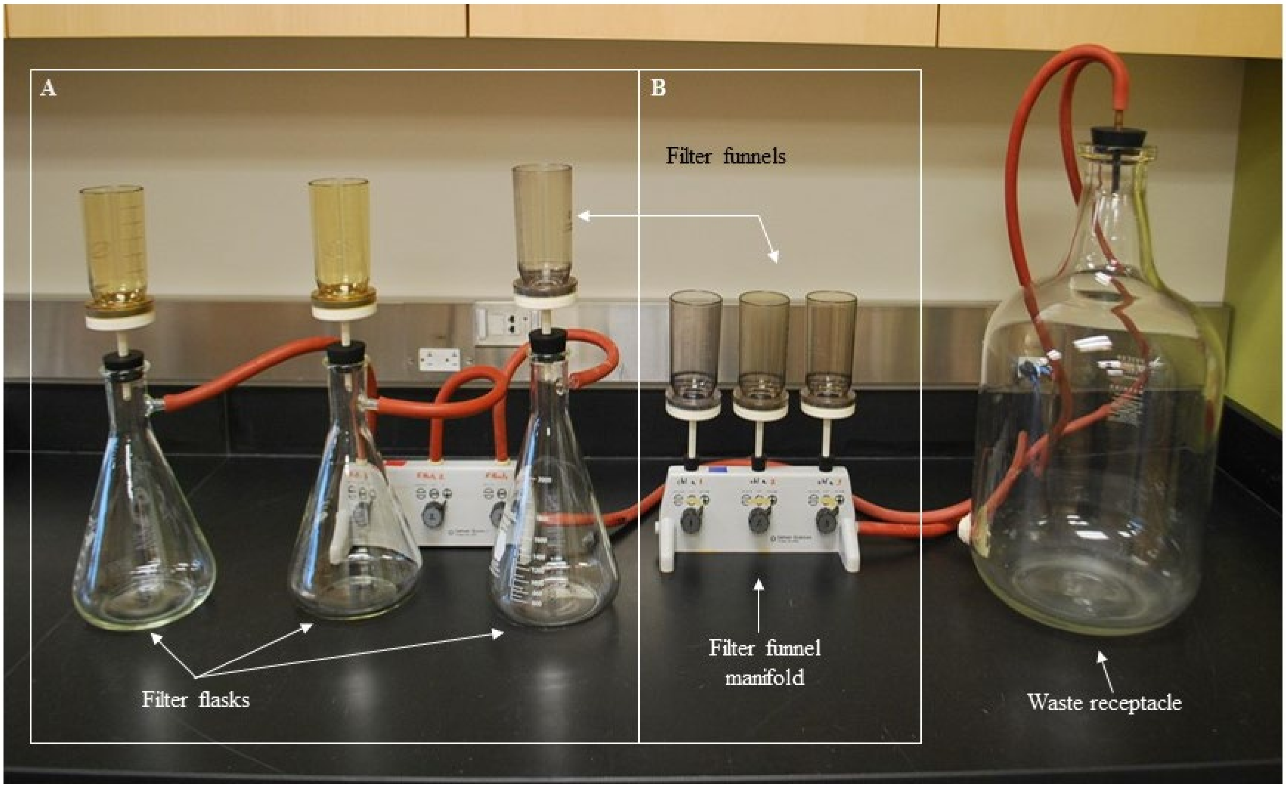

Figure 4.–Vacuum filtration configurations. Apparatus A shows the configuration for sequestering filtrate and isolating particulate organics. Apparatus B shows the configuration for isolating chlorophyll *a*.

Zooplankton data sheet - Kodiak Island Limnology Lab

Lake: **Station:** **Tow date:** **Tow depth (m):** **Net area (m):**

Zoo reader: **Zoo count #:** **Read date:** **Dilution (mL):**

read tally

Zooplankton	**1**	**2**	**3**	**4**	**zoo total**
read total:				**mean:**	

organism:			organism:			organism:			organism:			organism:		
#	length (mm)	length ovig (mm)	#	length (mm)	length ovig (mm)	#	length (mm)	length ovig (mm)	#	length (mm)	length ovig (mm)	#	length (mm)	length ovig (mm)
1			1			1			1			1		
2			2			2			2			2		
3			3			3			3			3		
4			4			4			4			4		
5			5			5			5			5		
6			6			6			6			6		
7			7			7			7			7		
8			8			8			8			8		
9			9			9			9			9		
10			10			10			10			10		
11			11			11			11			11		
12			12			12			12			12		
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15			15			15			15			15		
16			16			16			16			16		
17			17			17			17			17		
18			18			18			18			18		
19			19			19			19			19		
20			20			20			20			20		
21			21			21			21			21		
22			22			22			22			22		
23			23			23			23			23		
24			24			24			24			24		
25			25			25			25			25		

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Figure 5.–Zooplankton Data Sheet.

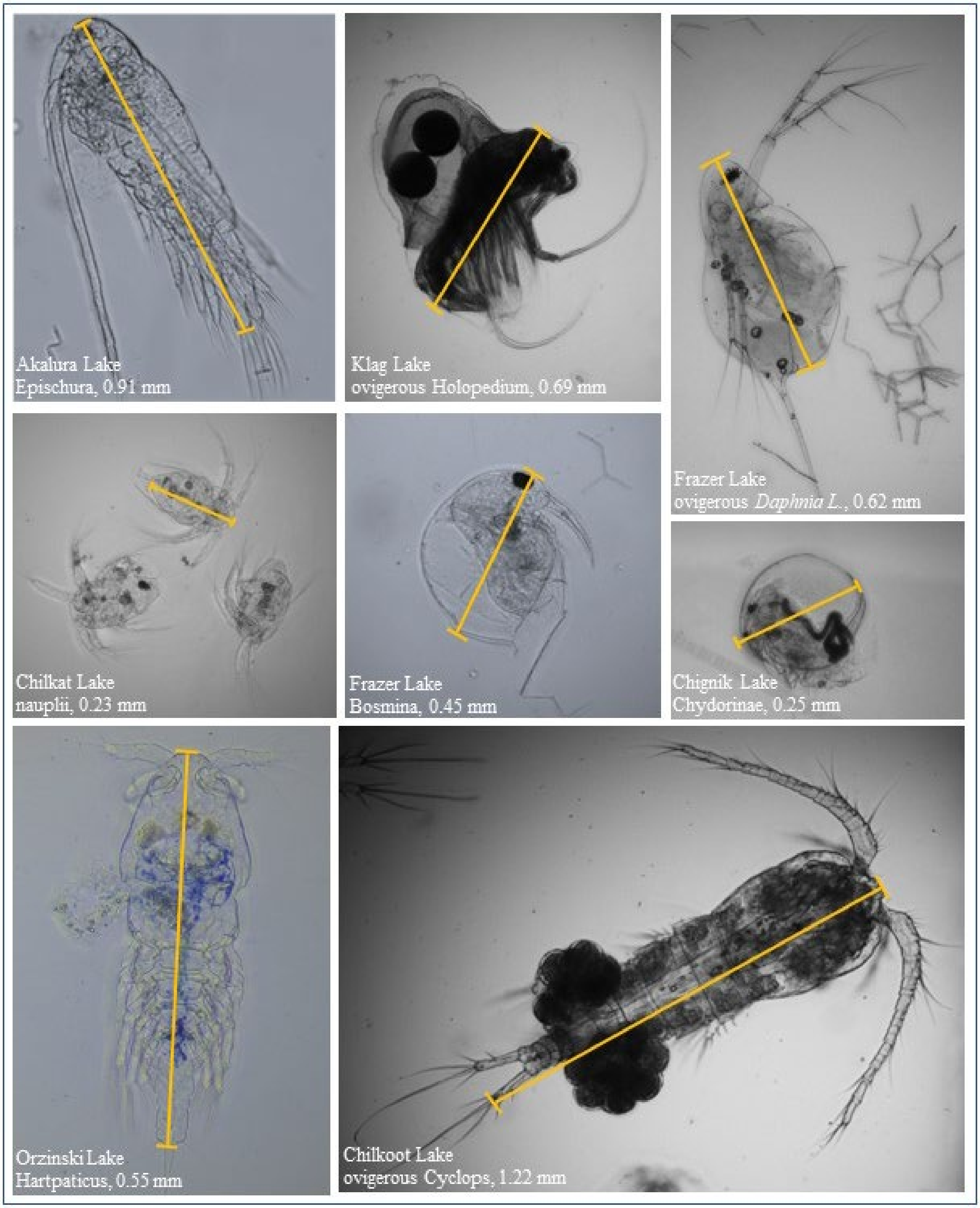


Figure 6.–Common zooplankton found in lakes of the Kodiak Island Archipelago, the Alaska Peninsula, and Southeast Alaska. Location, Genus, and size defined in each photo taken on a compound microscope at 200x. Ovigerous individuals have egg sacs. Nauplii measurement shown for scale; they are counted but not measured.

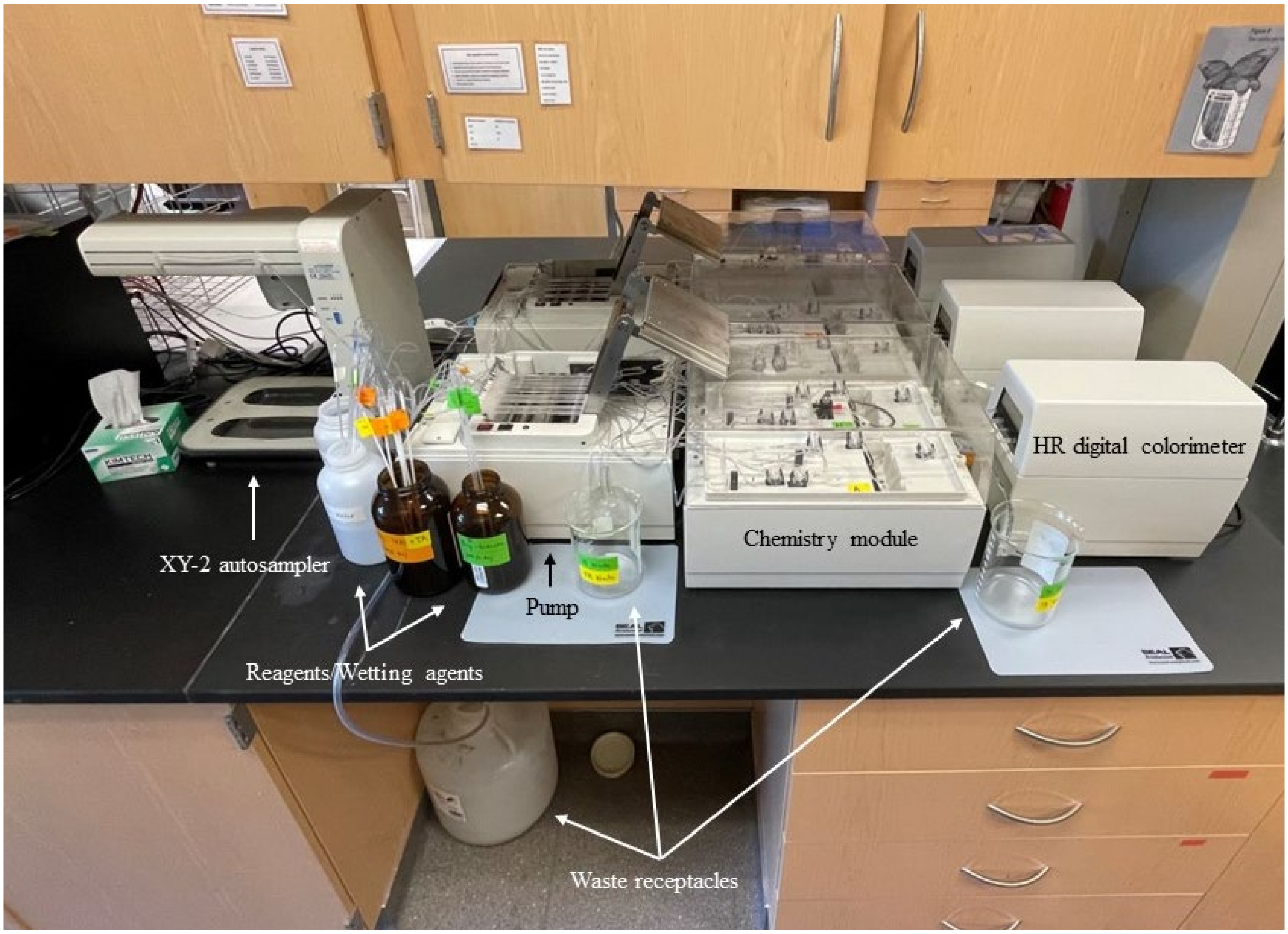


Figure 7.–SEAL Analytical AutoAnalyzer 3 (AA3) components. The Kodiak Island Limnology lab AA3 includes a computer with SEAL analytical software (not pictured), an XY-2 autosampler, two pumps, three chemistry modules, and three high resolution (HR) digital colorimeters.

APPENDIX A. SAMPLE PROCESSING AIDES

Appendix A1. –Kodiak Island Limnology Lab 2024 sample processing and contractor contact list.

Contact	Processing/Analysis	Institution	Mailing Address	Email Address	Phone Number
Jared McCrum Alan Heyvart	Total Kjeldahl Nitrogen	Desert Research Institute	2215 Raggio Parkway Reno, NV 89512	alan.heyvaert@dri.edu	(531) 318-4565
Genevieve Rich	Water Analysis Contract	Kodiak Regional Aquaculture Association	104 Center Ave #205 Kodiak, AK 99615	rm.limno@kraa.org	(907) 486-6555
Renae Ivanoff John Wade	Water Analysis Contract	Norton Sound Economic Development Corporation	PO Box 358 Nome, AK 99762	rinvanoff@nsedc.com jwade@nsedc.com	(907) 443-2477
John Beaver	Phytoplankton	BSA Environmental Services Inc.	23400 Mercantile Road, Suite 8 Beachwood, OH 44122	j.beaver@bsaenv.com	(216) 765-0582
Andy Piston	Water Analysis Contract	Alaska Department of Fish and Game	2030 Sea Level Dr. #205 Ketchikan, AK 99901	andrew.piston@alaska.gov	(907) 225-9677
Shelby Flemming Nicole Zeiser	Zooplankton Contract	Alaska Department of Fish and Game	PO Box 330 Haines, AK 99827	shelby.flemming@alaska.gov nicole.zeiser@alaska.gov	(907) 766-2830
Jenni Morella	Zooplankton Contract	Alaska Department of Fish and Game	401 Railroad Ave. Or PO Box 669 Cordova, AK 99574	jennifer.morealla@alaska.gov	(907) 424-3212
Sarah Laske	Chlorophyll *a*	U.S. Geological Survey	4210 University Dr. Anchorage, AK 99508	slaske@usgs.gov	(907) 789-7419
Dean Day Emily Heale	Water Analysis Contract	Cook Inlet Aquaculture Association	40610 K Beach Road Kenai, AK 99611	dday@ciaanet.org eheale@ciaanet.org	(907) 283-5761
Kyle Rosendale	Water Analysis Contract	Sitka Tribe of Alaska	204 Siginaka Way Sitka, AK 99835	kyle.rosendale@sitkatribe-nsn.gov	(907) 747-7182
Ashley Bolwerk Calvin Borges	Water Analysis Contract	U.S. Forest Service	2108 Halibut Point Road Sitka, AK 99835	ashley.bolwerk@usda.gov calvin.borges@usda.gov	(907) 747-4299

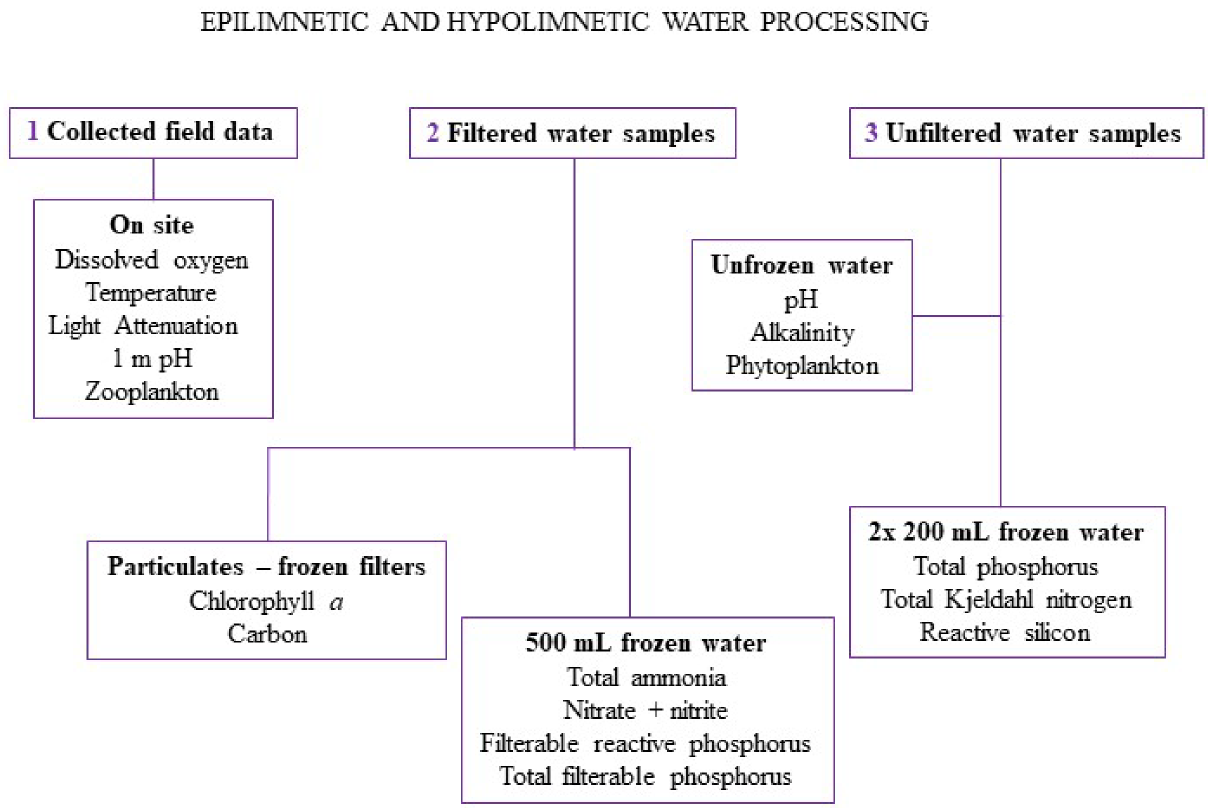
Appendix A2.–Field sampling equipment list.

Task or measurement	Field sampling equipment
Station set up	Sandbags (3 per station)
	Buoys (3 corkers and 1 inflatable per station)
	Steel 2 ft rod
	Ample (>100 m) 1/4" poly line
	Shovel
Water sampling	4 L Van Dorn sampler, messenger, and reel with 55 m of 1/4" metered poly line
	9 L Carboys (1 per station and sampling depth)
	500 mL sample bottles (for pH)
Zooplankton sampling	Plankton net with 200 mL dolphin bucket
	Reel with 55 m of metered 1/4" poly line
	Cannonball weight to test for bottom
	125 mL Nalgene bottle with 10 ml of formalin (1 per station)
	DI squirt bottle
Physical parameters	ProODO meter
Temperature and dissolved oxygen	Sensor and 50 m cable marked in meters
	2 spare C batteries
Light	Li-Cor 250A Light meter
	Underwater Quantum sensor and 50 m metered cable
	Spare 9V battery
pH	YSI pro10 pH meter
	pH sensor and cable marked at 1 m
	2 spare C batteries
Water clarity	Secchi disk
Temperature monitoring	HOBO shuttle
	Spare HOBOs
	1 m deployment lines with halibut snaps
Miscellaneous	Clipboard
	Rite in the Rain Detailed Lake Survey forms
	Hand-held GPS with lake maps
	Sharpies and pencils
	Electrical tape
	Multitool
	Electrical tape
	Survival kit
	Personal flotation device

Appendix A3.–Kodiak Island Limnology Lab field temperature logger sites. The Alaska Department of Fish and Game assists the Kodiak Regional Aquaculture Association (KRAA) with additional sites during summer sampling.

Project	Site	Logger types	Season start	Season end
Temperature Monitoring Network	Afognak Lake	Water	May	Overwinter
	Akalura Lake	Water	May	Overwinter
	Ayakulik weir	Water	May	Overwinter
	Buskin weir	Water	May	Overwinter
	Dog Salmon weir (lower)	Water, air	May	Overwinter
	Frazer weir	Water, air	May	Overwinter
	Karluk weir	Water, air	May	Overwinter
	Paul’s Bay weir	Water, air	May	September
	Uganik Lake	Water, air	May	September
	Upper Station weir	Water, air	May	Overwinter
KRAA	Buskin Lake	Water, array	May	Overwinter
	Crescent Lake	Water, air	May	September
	Hidden Lake	Water	May	September
	Little Waterfall Creek	Water, air	May	Overwinter
	O’Malley Lake	Water	May	September
	Saltery weir	Water, air	May	September
	Spiridon Lake	Water	May	September
	Telrod Creek	Water, air	May	September
	Thumb Lake	Water	May	September
	Upper Malina	Water, air	May	September
	Upper Olga	Water, array	May	September

Appendix A4.–Lake water processing flow chart.



Appendix A5.–AA3 monthly system cleaning procedure.

System clean

1 N HCL	10 minutes
DI water	10 minutes
1 N NaOH	10 minutes
DI water	10 minutes
10% bleach	10 minutes
DI water	10 minutes

AA3 monthly maintenance

1 Clean and lubricate pump rollers (2 drops oil on each pad)
2 Clean and lubricate side rails (semi-fluid lubricant)
3 Clean pump platen (isopropyl alcohol swab)
4 Clean sampler wash pot (isopropyl alcohol swab)
5 Remove or replace PharMed tubing
6 Full system clean

System clean solutions

	500 mL DI water	1 L DI water
1 N HCL	41.25 mL concentrated HCL	82.5 mL concentrated HCL
1 N NaOH	20 g NaOH pellets	40 g NaOH pellets
10% bleach	50 mL concentrated bleach	100 mL concentrated bleach

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Appendix A6.–Example serial dilution calculator for phosphorus (P) nutrient chemistries.

Total P, Total Filterable P, and Filterable Reactive P as P

H_2KPO_4 mass	136.09	g/mol
P mass	**30.97**	**g/mol**

P stocks:

1° stock

H_2KPO_4	**0.2197**	**g**
final volume	500	mL
[P]	**100.01**	**mgP/L**
	100007	µgP/L

low concentration quality conctrol

	µgP/L	10% range		15% range	
inorganic P	**4.24**	3.82	4.66	3.60	4.88
organic P	**6.11**	5.50	6.72	5.20	7.03

2° stock

1° stock	**2.5**	**mL**
final volume	250	mL
[P]	**1.00**	**mgP/L**
	1000	µgP/L

standards: serial dilution volume 100 mL

flask	**volume 2° stock**	desired [P]		
#	**mL**	µgP/L	mgP/L	µM
901	**3.00**	30	0.030	0.97
902	**2.00**	20	0.020	0.65
903	**1.00**	10	0.010	0.32
904	**0.50**	5	0.005	0.16
905	**0.20**	2	0.002	0.06
906	**0.10**	1	0.001	0.03

flask	volume 2° stock	**final [P]**		
#	mL	**µgP/L**	mgP/L	µM
901	3.00	**30.002**	0.030	0.97
902	2.00	**20.001**	0.020	0.65
903	1.00	**10.001**	0.010	0.32
904	0.50	**5.000**	0.005	0.16
905	0.20	**2.000**	0.002	0.06
906	0.10	**1.000**	0.001	0.03

standards: serial dilution volume 25 mL

flask	**volume 2° stock**	desired [P]		
#	**mL**	µgP/L	mgP/L	µM
901	**0.75**	30	0.030	0.97
902	**0.50**	20	0.020	0.65
903	**0.25**	10	0.010	0.32
904	**0.12**	5	0.005	0.16
905	**0.05**	2	0.002	0.06
906	**0.02**	1	0.001	0.03

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APPENDIX B. SEAL AA3 OPERATING NOTES

1. For seawater analysis use artificial seawater. For water and wastewater analysis use DI water as sampler wash solution. *See also operating note 2.*

2. Recommended procedures for best performance when analyzing low concentrations:

   - Pure water may be double distilled water (DDW) or DI water. For DDW, the analyst must be careful to avoid contamination with silicic acid from dissolution of glass.
   - For accurate low concentration work, all glassware used for making reagents should be acid washed. Store flasks "shaken dry" and capped instead of leaving them to dry on the counter if worried about contamination.
   - Sample cups must be clean. Acid wash before use. When pouring samples, rinse once with sample or standard solution.
   - Use plastic bottles for sample storage or transport. High density polyethylene or polypropylene bottles are acceptable, but do not use glass. Glass contaminates the samples with silicic acid.
   - Samples should be measured as soon as possible after sampling.
   - Skin contact must be avoided with anything which will touch the reagents and samples. Wear gloves. Avoid ammonia contamination in the air (e.g. by smoking, farmyard, industrial smoke or vapor, other reagents or strong odors).
   - The laboratory temperature should be stable, with no strong air currents around analyzer. Run the system with the manifold cover in place.
   - Rinse the manifold according to operating note 8. Rinse wash receptacle each day by pumping baseline reagents for 15 minutes before starting a run. Clean the wash receptacle once a month with hypochlorite solution.
   - The volume between the air valve and the injection fitting should be minimal, using 0.015" polyethylene tubing cut short. The joints between glass parts cannot contain gaps.
   - A regular bubble pattern is necessary for low noise. If the bubble pattern is irregular, check that all plastic tubing is correctly wetted (bubble shape round at front and back. After replacing the pump tubes or parts of the manifold, pump 1 N NaOH through all tubes for 15 minutes (also see operating note 15).

3. If running only in the lowest range, connect the color reagent to the line for sample B and tie off the line for the color reagent. The baseline noise, and thus the detection limit, can then be further reduced by diluting the reagents by a factor of two or five. The linearity of the used range must be checked. The high amount of EDTA in the complexing reagent is only necessary for seawater samples containing high concentrations of calcium and magnesium ions and when using the yel/yel sample pump tube. At lower calcium and magnesium concentrations, or when using the orn/grn sample pump tube, the EDTA concentration can be reduced to 15 g/L.

4. Final pH: For optimum results the pH of the final reaction must lie within 11.5 and 11.9. Collect the solution from the flowcell waste line to check the pH. If the final pH is too high, reduce the sodium hydroxide concentration by mixing in phenol solution. If the pH is too low, add sodium hydroxide to the phenol. Note how much of each was added on the run sheet.

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5. Ammonia is a common contaminant in the atmosphere and the environment. Take extra precautions to avoid contamination of the reagents. Do not touch any surfaces which will be in contact with reagents or samples. Check the reagent absorbance each time fresh reagents are made: if too high, the detection limit will increase. Rinse sample cups with sample before filling.

6. Manifold cleaning procedure: Every day, use system wash solution (2 mL/L Brij-35, 15% solution). Once a week, or when a precipitate is visible in the coils, pump special wash solution (1 N HCl) through the system and the sample line for 10 minutes, then the system wash solution for 30 minutes.

7. If dual-range operation is not needed, remove sample line B and tie off or remove the T-piece.

8. If dual-range operation is needed, pump DI water through the sample line which is not connected to the sample probe.

9. Wavelength Filter: Phenol reagent 630 nm.

10. If nitrate is not used on this multitest cartridge, remove the polyethylene tubing between the C3 and the A10 fitting and connect directly using a sleeve made of 116-0536-16 tubing.

11. Concentration of free chlorine reagent. The concentration of free chlorine in the reaction mixture is critical to correct sensitivity and linearity. As neither the source chemical nor the solution are stable, the concentration should be adjusted by experiment if the method becomes non-linear or the sensitivity is low. Baseline noise and drift will be optimized by using the lowest concentration which gives acceptable results.

12. If DCI is not readily available, sodium hypochlorite can be used as an alternative chlorine source. In most cases, diluting the stock solution 10:1 with water will produce an acceptable working reagent whose concentration should then be optimized by experiment. Reagent optimization should be repeated every few months or when a new bottle of chemical is opened. If using NaOCl, which is alkaline, check the pH after optimizing the concentration of the NaOCl.

13. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (the trailing edge of the bubbles is rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting.

14. If the bubble pattern out of the heating bath becomes irregular, it may help to change the second air tube from blk/blk to orn/orn.

1. For seawater analysis use a sampler wash solution containing sodium chloride and sodium hydrogen carbonate if the method will be run at 60 samples per hour. Use DI water in water and wastewater analyses. It is possible to use DI sampler wash solution for seawater analysis, but the sampling rate should be reduced because there will be a disturbance at the beginning and end of each peak. The peak window on the AACE Software is then programmed to ignore the disturbance.
2. For the most accurate results, the standard diluent should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from silicate content in the inorganic salts used for artificial seawater, we recommend using a 0 calibration standard of low-nutrient seawater of known low concentration.
3. Recommended procedures for best performance when analyzing low concentrations can be found under operating note 2 of the TA in Water method.
4. The nitrite concentration can be determined by eliminating the reductor column and standardizing with an appropriate nitrite solution. To determine the nitrate values, the nitrite alone must be subtracted from the total (N+N). Check the efficiency of the reductor column regularly by analyzing equi-molar N+N standards. The reduction efficiency should be >95%.
6. Start-up procedure:
   a. Check the reagent volumes to ensure an adequate supply and ensure the valve to the cadmium redactor column is closed.
   b. Place reagents on-line and start the pump.
   c. When the ammonium chloride reagent has reached the end of the C3 fitting, connect the reductor column by using the 4-way valve, being careful not to get any air bubbles into the column.
7. Shut-down procedure:
   a. Stop the pump and disconnect the reductor column by closing the 4-way valve.
   b. Start the pump, remove all reagent lines and place in their respective wash solutions
   c. After 10 minutes stop the pump and remove the platen.
8. Reagent Background Color
   a. Pump DI water containing 1 mL/L of 15% Brij-35 through the system.

      NOTE: The cadmium column should not be connected at this time.
   b. Follow the start-up procedure (operating note 6).
   c. The absorbance reading of the reagents compared to DI water should not be more than 0.04 (4%). If the absorbance is greater than six units, a reagent or the water used to make the reagents is likely contaminated.
9. To extend the column life between regeneration, use N instead of air for the segmenting bubbles.
10. Brij-35. Old or oxidized Brij-35 causes poor peak shape and low recovery. Use pellets to make fresh 15% Brij-35 (Common Use Solutions; page 14).

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11. Additional performance data:

    Sample A - Sample B

    Lag time 6 to 7 minutes

    Carryover 0.3%

    a. If dual-range is not needed, remove sample line B and tie off or remove the T-piece.
    b. Pump DI water through the sample line which is not connected to the sample probe.
    c. Manifold cleaning procedure: everyday, use system wash solution. Once a month, wash the manifold, including the sample line, with diluted hypochlorite solution containing 0.5 mL of Brij, after disconnecting the cadmium column. Rinse with system wash solution.
    d. For total N, prepare a digestion reagent (Grasshoff, 1983) as follows: 15 g H_3BO_3, 25g $K_2S_2O_8$, 7.5 g NaOH diluted to 500 mL with water. Prepare the digestion reagent fresh weekly. To digest samples, add 5 mL reagent to 50 mL sample in a suitable PTFE pressure bottle. Heat at 115º C for two hours. The pH of the digested samples should be ~8.

12. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and wetted tubing (the trailing edge of the bubbles must be rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting.

13. If the bubble pattern out of the heating bath becomes irregular, the size of the second air injection pump tube may be increased from blk/blk to orn/orn.

1. For seawater analysis use sampler wash solution containing sodium chloride, sodium hydrogen carbonate and sulfuric acid. For water and wastewater analysis use only DI water with sulfuric acid. The acid concentration should be the same as in the samples. Because samples lose acid during digestion, titrate a sample to determine the actual acidity.

2. The standard diluent must have the same matrix as the sampler wash solution. Therefore, use acidified artificial seawater for seawater analysis. Constant acidity between samples and standards is critical to correct performance. For most accurate results digest the standards.

3. This method runs on multitest MT19 using the high range only. It cannot be run on the low range using the large sample pump tube due to insufficient acid neutralization capacity in the reagents (see operating note 12).

4. Low reagent absorbance is critical to the correct operation of this method. Check the reagent absorbance daily and if it is higher than specified, use purer reagents, take extra steps to guard against contamination or boil the buffer solution.

5. Check the pH of the final reaction mixture arriving at the flowcell with a digested sample pumping through the sample line. The pH should be 12.8–13.0. If the pH is higher than 13.0 the concentration of sodium hydrogen phosphate-12-hydrate must be increased. If the pH is lower than 12.8 the concentration of sodium hydroxide in the buffer solution must be increased. The method is designed for samples containing 4% v/v H_2SO_4. If samples with different acidity are measured, the buffer must be adjusted accordingly.

6. Place the salicylate reagent line in its container last and remove it first to avoid precipitation of reagent in the manifold when the acid sample wash solution is present.

7. The concentration of free chlorine is critical for optimum sensitivity and linearity. If necessary, adjust the concentration of free chlorine by varying the concentration of the dichloroisocyanuric acid sodium salt dihydrate. Finally recheck the pH. Hypochlorite solution may be used as an alternative chlorine source. However, it is unstable and the optimum concentration must be established regularly. As the solution is alkaline, pH adjustment may be required.

8. Use a Kel-F, PEEK, or platinum sample probe.

9. Additional Performance Data

Lag time	8 min
Carryover	<0.4 %

10. Performance with AAII colorimeter and 15 mm flowcell

Due to the difference in flowcell path length, some parameters will be different:

Sensitivity: Extinction at 5.6 mg N/L	0.32–0.40
Reagent absorbance	0.02–0.05

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11. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (the tailing edge of the bubbles must be rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting.
12. Method range 0–2.2 mg N/L uses pump tubing org/grn (0.10 mL/min). To perform range 0–700 µg N/L determinations install a pump tubing blk/blk (0.32 mL/min) to aspirate the sample. The higher flow rate of the acidified sample causes a higher acidity in the flow stream and therefore the sodium hydroxide concentration of the buffer should be increased to 30.5 g/L. Check the pH according to operating note 5.

1. For low concentration work (0–50 µg P/L), 2 pump tubes may be exchanged to achieve better sensitivity (0.025 µg P/L):

   Sample: replace yel/yel (1.20) with blu/blu (1.60)
   Water 1: replace orn/orn (0.42) with orn/grn (0.10)

   If tubes are changed, the manifold is no longer suitable for TA or N+N, which needs a higher reagent: sample ratio.

2. Recommended procedures for best performance when analyzing low concentrations can be found under operating note 2 of the TA in Water method (Page 51).

3. Manifold cleaning procedure: Every day, pump system wash solution (8 g/L sodium dodecyl sulfate) through all reagent lines.

   Every month, pump special wash solution (20% hypochlorite) for 20 minutes through the system and the sample line, then pump system wash solution for 30 minutes.

4. If the orthophosphate chemistry is to be used following a chemistry that uses Brij-35 as wetting agent (e.g. N+N), wash thoroughly with 1 N H_2SO_4 for 10 minutes, pumping wash solution for 15 minutes, and then connect the reagents.

5. Additional performance data.

   Sampler to spectrophotometer time: 7 minutes, 5 seconds

   Carryover: 0.5 %

6. The solubility of potassium persulfate in water is 4.49 g/100 mL water (at 20° C). The digestion reagent is purposefully over saturated and therefore residual potassium sulfate will remain out of solution. Avoid the residual potassium persulfate when transferring the reagent to the storage bottle.

Appendix B5.–Silicon operating notes.

1. For accurate results, the standards should have the same matrix as the samples. To avoid errors from Si content in the inorganic salts used for artificial seawater, use a zero-calibration standard of low nutrient concentration seawater.
2. Recommended procedures for best performance when analyzing low concentrations can be found under operating note 2 of the TA method (page 51).
3. Additional performance data

Sample A	Sample B	
Carryover at 60/hour	0.3%	0.3%
Lag time	7 minutes	10 minutes

4. Manifold cleaning procedure:

   Every day, use system wash solution (2 g/L sodium dodecyl sulfate). Every month, pump special wash solution (20% hypochlorite) through the system and the sample line for 20 minutes, then system wash solution for 30 minutes.

5. Use 0.03” polyethylene tubing for the sample transmission line if running the method alone, otherwise tubing of a suitable diameter for the total flow rate.
6. If dual-range is not needed, remove sample line B and tie off or remove the T-piece.
7. Pump DI water through the sample line which is not connected to the sample probe.
8. If nitrate is not used on this multi-test cartridge, remove the tubing between the C3 and the A10 fitting and connect directly using a sleeve made of 116-0536-16 tubing.
9. If the silicate chemistry is to be used following a chemistry that uses Brij-35 as wetting agent (e.g. N+N), wash thoroughly with 1 N H_2SO_4. Brij-35 interferes with the silicate chemistry.
10. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (trailing edge of the bubbles must be rounded). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting.
11. If the bubble pattern out of the heating bath becomes irregular, the size of the second air injection pump tube may be increased to orn/orn.
12. The oxalic acid reagent is meant to be a saturated solution. The solubility of oxalic acid in water is 90–100 g/L so residual oxalic acid may remain in the flask. Put a wand filter on the end of the oxalic acid reagent wand to negate potential interference from undissolved oxalic acid.
13. The serial dilution calculator describes the concentration of Si in its elemental form while this method describes the concentration of the silicate compound SiO_2. The lower concentration range of this method is 0–6 mg/L SiO_2, which is equivalent to ~ 0–2.8 mg/L of Si. When creating a standard curve, do not exceed 4000 ug Si/L as a high standard. If samples are outside of this range, perform a serial dilution until sample concentrations are within the method range, and back calculate to determine the total concentration.