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Limnological Methods for the McMurdo Long Term Ecological Research Program

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Section 1 Introduction

Project Summary

The McMurdo Dry Valleys (MCM) region is among the most extreme deserts in the world: far colder and drier than any other LTER site. The MCM contain a mosaic of perennially ice-covered lakes, ephemeral streams, glaciers and exposed soils, all of which contain viable biological communities capable of surviving the extreme climatic conditions, particularly the lack of liquid water. Subtle changes in climate have a major influence on the generation of liquid water, which produces a cascade of processes influencing the productivity, biodiversity and biogeochemistry within MCM.

MCM-I focused on the role of physical constraints on the structure and function of the ecosystem. Our research indicated that small changes in temperature and albedo are amplified by large non-linear changes in hydrological cycle that propagate through the ecosystem. The central hypothesis of MCM-II was that the legacy, or memory of past climate change upon the landscape, strongly overprints present ecological conditions in MCM. These legacies include ancient lacustrine organic matter now available to the soil ecosystem and concentrated, ancient nutrients in the lakes that support present day phytoplankton productivity. Results obtained in MCM-II further implied that biodiversity and related ecosystem processes are controlled by a balance between legacies of past climates and contemporary processes. Research in MCM-III continued to investigate the MCM as a climate sensitive "end-member" ecosystem, and began to focus on the roles of legacy and extant process on contemporary biodiversity and ecosystem structure and function. MCM IV intends to focus on the hypothesis that climate warming in the McMurdo Dry Valley ecosystem will amplify connectivity among landscape units, leading to enhanced coupling of nutrient cycles across landscapes, and increased biodiversity and productivity within the ecosystem.

In this proposed research, contemporary patterns in ecological connectivity in the MDV will be examined and used as a basis for predictions of future changes. Warming in the MDV is hypothesized to act as a slowly developing, long-term press of warmer summers, upon which transient pulse events of high summer flows and strong katabatic winds will be overprinted. Four specific hypotheses address the ways in which pulses of water and wind will influence contemporary and future ecosystem structure and function. Dramatic increases in connectivity are projected to occur in the next century, e.g. the closed basin lakes of Taylor Valley will likely rise and may begin to coalesce. To better understand this scenario, the MCM LTER project will expand to include the more southerly Miers and Garwood Valleys, where thermokarst erosion is already underway. Because windborne transport of biota is a key aspect of enhanced connectivity from katabatic winds, new monitoring will include high-resolution measurements of aeolian particle flux. Importantly, integrative genomics will be employed to understand the responses of specific organisms to the increased connectivity. The project will also include a novel social science component that will use environmental history to examine interactions between human activity, scientific research, and environmental change in the MDV over the past 100 years. In much the same way as the simplicity of the MDV ecosystems makes the area an ideal location for exploring ecological theory, the simplicity of the area's human history – in terms of its short timeframe and the small number of people involved – makes it an excellent location for integrating the theory and practice of environmental history with the ecological research of the LTER network.

Section 2. Field Procedures
2.1 Sampling Sites

Location of Sampling Sites

Limnological sampling sites are located in an area of each lake that corresponds to the deepest portion of the lake. A "Blue Instrument Box" on the ice surface is close to these locations. The Blue Box contains Campbell data loggers that collect and store annual underwater photosynthetically available radiation (PAR) at 10 m below the piezometric water level, incident PAR, and lake ice temperature data. In addition, there are two pelagic sediment trap arrays (flagged) located near the Blue Box. The exact location of each sample hole varies from year to year and is dependent on sampling hut (Weatherport/Polarhaven) construction. Every year the NSF contractors are provided with a map indicating the ideal location (within a 15 - 30 m radius of sediment traps) to construct the sampling huts (Figure 1). However, the location typically depends on lake ice topographical features. Once the huts are constructed, the sampling holes are drilled. GPS locations are taken at the sampling holes on each lake each year using Garmin 12XL units. Two limnological sampling holes are located at each site, one inside the hut for sample collection, and one outside the hut (~20 m away) for *in situ* primary production incubations and instrument data collections. These two holes are the primary limnological sampling locations used throughout the season for replicate data collection and receive the designation of "Limnological Transect 1" (i.e., Hor LT1, Frx LT1). For the east and west lobes of Lake Bonney, the primary limnological sampling holes have been designated as Bon E30 and Bon W20, respectively. This nomenclature follows a code developed by J. C. Priscu for synoptic sampling (Spigel and Priscu, 1998). The holes located over the deepest portion of each lobe correspond to Bon E30, and Bon W20. In addition, secondary sampling holes may be drilled in other areas of the lake to address specific limnological hypotheses. For lakes that may be sampled once a year (i.e., Lakes Joyce, Miers, Trough, and Vanda) a centralized area has been identified with GPS coordinates, and efforts to collect in the same location are made every year. These sampling locations have been identified as Joy LT1, Mer LT1, Tro LT1, and Van LT1.

A 4 inch Jiffy ice auger is used to drill both sampling holes. In the early part of the season (October) it is important not to penetrate the ice cap completely with the auger. Drill down to a depth of about ~1 m above the bottom of the ice, this will prevent the auger from freezing into the hole. Once the hole is complete, use the hole melter (a modified steam cleaner with a heated glycol loop) to penetrate the ice cap and melt the hole to a diameter of ~ 50 cm. Periodic melting of the hole will be necessary to keep the hole open the entire season. Later in the season, the Jiffy augers may be used to penetrate the ice cap without freezing in the bit and flight extensions.

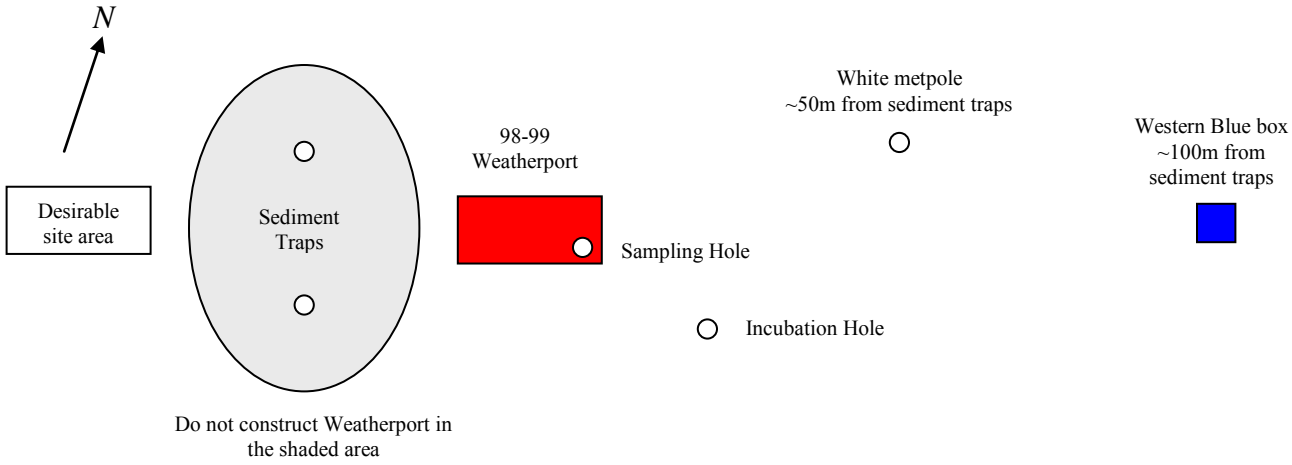
Materials

4 and10 inch Jiffy flight extensions and cutting bits
5 kw generator
Flight extension bolts
Hole melter (Hotsy Models)
Jiffy ice auger powerhead
Standard allen wrench set
Standard socket set

Figure 1. Construction of Weatherports at LTER sampling sites

Weatherports should be >15 m but <30 m from sediment trap holes, on a low flat area if possible. The shaded are in the figures below represents the <15 m zone. The sediment trap holes are identified with flagged markers and a GPS location is provided.

East Bonney Weatherport location: **Note**: As of November 1998 there are two blue boxes on East Lobe Bonney. The LTER project operates the western most box; A LExEn Project (J. C. Priscu Co-PI) operates the eastern most box. The GPS location for East Bonney sediment traps is: *S* 77° 42.837', *E* 162° 26.619'.



West Bonney Weatherport location: The GPS location for West Bonney sediment traps is: *S* 77° 43.183', *E* 162° 17.915'.

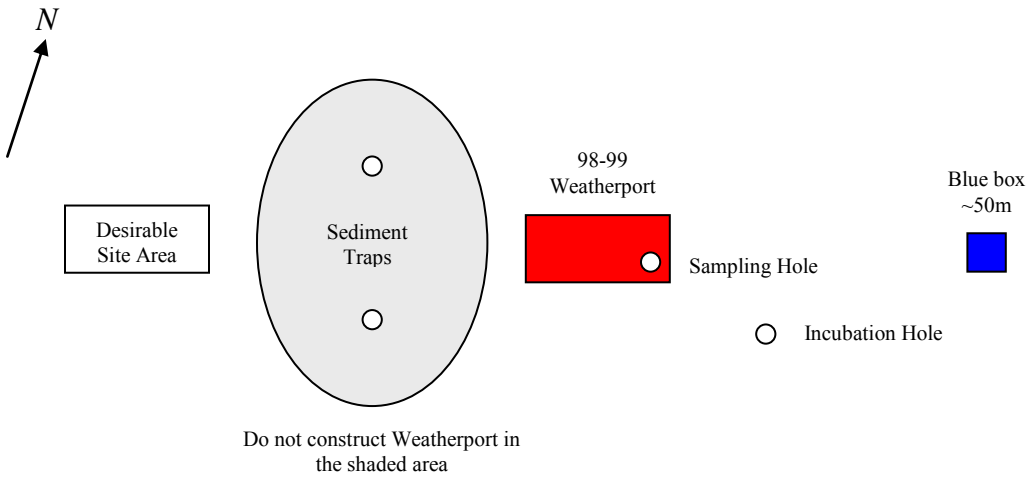
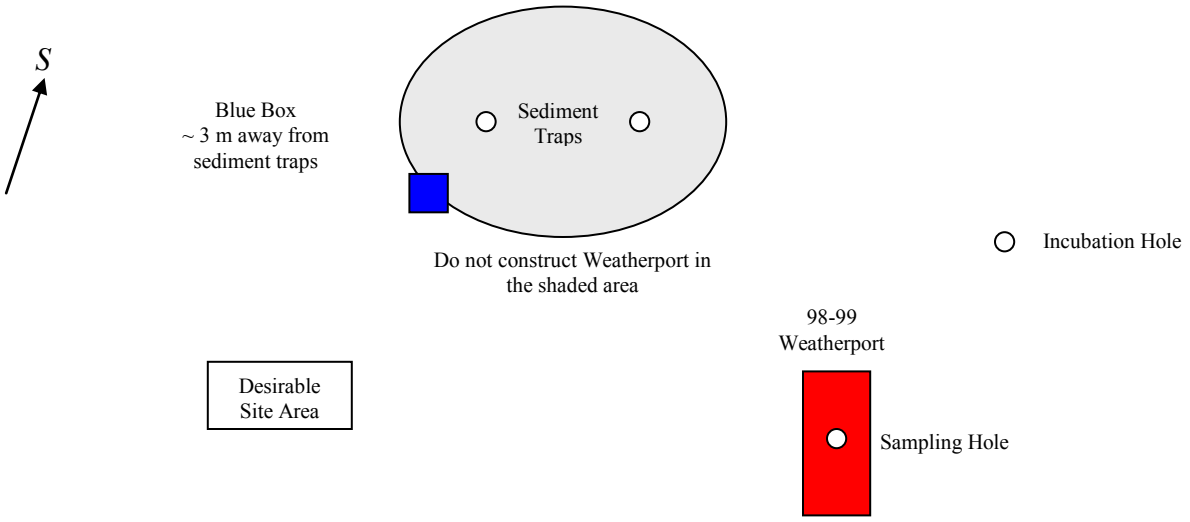
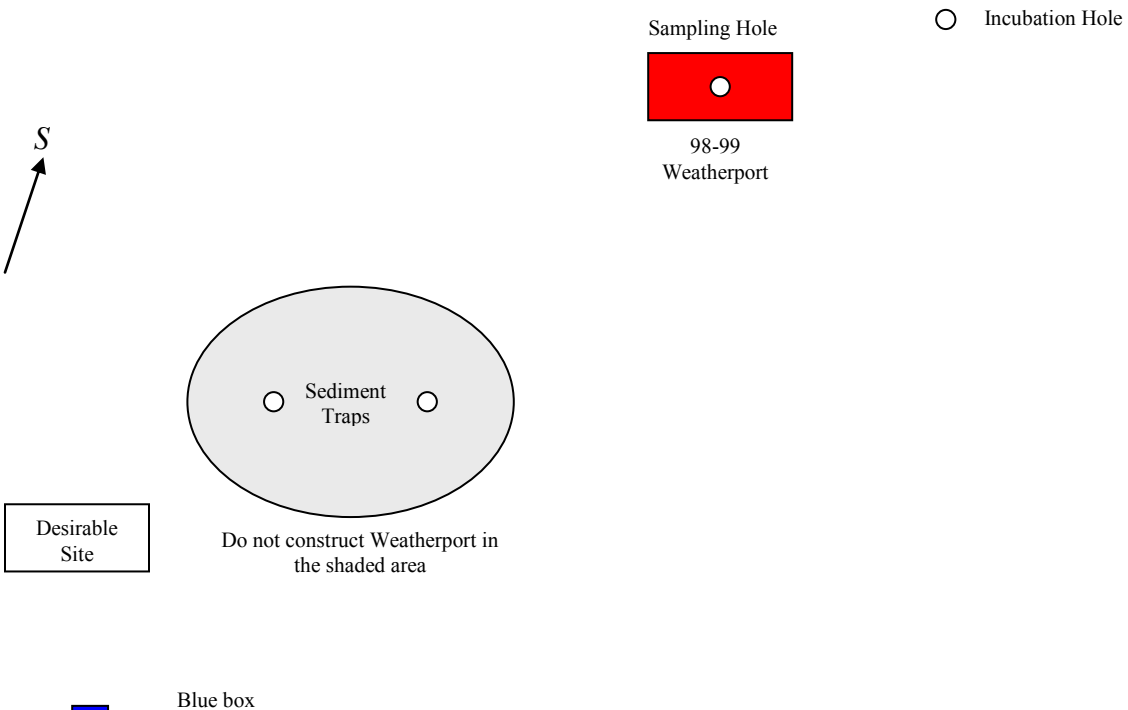


Figure 1. (continued)

Lake Fryxell Weatherport location: The GPS location for Lake Fryxell sediment traps is *S* 77° 36.619' *E* 163° 08.642'.



Lake Hoare Weatherport location: **Note**: Due to the "extreme" ice conditions on Lake Hoare during the 98-99 season, the Weatherport was located ~ 100 m away from the sediment traps. Unfortunately, this area was situated over a relatively shallow portion (depth = 18 m) of the lake, and data between 18 – 30 m was not collected. Since that time, the Weatherport has been located over similarly shallow areas over various years, and the bottom sampling depth has varied between 18 and 30m. The GPS location for Lake Hoare sediment traps is *S* 77° 37.699' *E* 162° 54.498'.



References

Spigel, R.H. and J.C. Priscu. 1998. Physical limnology of the McMurdo Dry Valley lakes. *In* Ecosystem dynamics in a polar desert: The McMurdo Dry Valleys, Antarctica, pp. 153-187, J.C. Priscu (Ed). American Geophysical Union.

Lake Water Sample Collection

General Discussion

The McMurdo LTER performs baseline limnological data collections on dry valley lakes to address unilateral LTER core areas of research. These core areas include primary production, trophic structure, accumulation of organic matter, nutrient cycling, and ecosystem disturbance. To address these core areas, the MCM LTER typically performs three replicate samplings (Limno Run) of Lakes Bonney (east and west lobe), Hoare, and Fryxell during the austral summer. Baseline analyses performed during each limno run include the following:

- Primary production (PPR)
- Chlorophyll-*a* (CHL)
- Phytoplankton [(PHYTO) enumeration and biomass]
- Profiling natural fluorescence (PNF)
- Bacterial production (TDR)
- Bacteria [(BAC) enumeration and biomass]
- Dissolved inorganic carbon (DIC)
- Dissolved organic carbon (DOC)
- Particulate organic carbon and nitrogen (CN)
- Particulate Phosphorus (PP)
- Macronutrients (NUT)
- Anions/Cations (AN/CAT)
- Conductivity, temperature, and depth (CTD)
- Dissolved oxygen (DO)
- Hydrogen Ion concentration (pH)
- Profile/Logged Photosynthetically active radiation (PAR)
- UW spectral fluorometry (FLUOROPROBE) (new 0405)

These analyses are performed at specific depths in each lake to capture important vertical characteristics. Appendix 6.1 lists the target depths at which samples are collected. However, due to environmental conditions (ice thickness, bottom depth in area of sample hole) these depths cannot always be sampled. We aim to collect the top sample at approximately 0.5m below the bottom of the ice cover; and the bottom sample as close to the bottom of the lake as possible without disturbing the sediment. This section discusses the collection and allocation of lake water for biological, chemical, and physical analyses.

Materials

Sampling Gear
- Bamboo poles (2)
- Bucket
- Chair
- Chipper bar
- Crazy creek chair
- Dishwashing gloves (insulated)
- Grey water carboy, 2 at Bonney
- Ice screws
- Silicone tubing for Niskin
- Li-Cor LI-1000
- Messenger (2)
- Niskin bottle (5 l)
- Niskin bottle stand
- Parachute cord
- Plastic funnel
- Sieve or net
- Rad tray with Benchcoat lining
- Table
- Tarpaulin (opaque)
- Weight for cable
- Winch stand
- Winch with pipes

Sample Bottles and Supplies
- 1000 ml HDPE bottles (1 clear, 1 amber for each depth)
- 500 ml HDPE amber bottles (Phytoplankton)
- Coolers (2-3)
- PPR bottles and transport carrier
- PPR incubation line
- Scintillation tray

Dissolved Inorganic Carbon
- P200 Gilson Pipetman and tips
- 30 ml serum bottles
- Aluminum seals
- Capper tool
- Chloroform (0.15 ml 30 ml sample^{-1})
- Rubber stoppers

Dissolved Oxygen (Winkler)
- P1000 Gilson Pipetman and tips (2)
- Alkline-iodide-azide reagent
- Manganese reagent
- Benchcoat
- Kimwipes
- Vinyl gloves (at least 3 pair)
- Bag for solid non-rad waste
- Scintillation vial for each sample
- Serum/scintillation vial transport carrier

pH
- Scintillation vial for pH sample

Radioisotopes

^{14}C bicarbonate (100-120 $\mu Ci\ ml^{-1}$)
P1000 Gilson Pipetman and tips (rad only)
Spreadsheet of isotope addition
Vinyl gloves (at least 3 pair per person)
Ziplock for solid rad waste

Procedure

Before a limno run, it is important to ensure that the sampling and incubation holes are completely opened and will allow all sampling instruments to pass through. Sampling equipment should be carefully examined for proper operation and the winch lines should be checked for proper depth calibration. The collection process and filtration of lake water for analyses may take up to 18 h to complete, therefore, it is important to start sampling by 5:00 am. Also, the primary production *in-situ* incubation should start no later than 7:30 am to maximize "daytime" PAR. Therefore, it is particularly helpful if all of the sampling equipment and sample bottles are prepared (cleaned and labeled) and arranged in the sampling hut the day before a limno run. This includes supplies and reagents used to fix the dissolved oxygen and dissolved inorganic carbon samples. At each depth, lake water samples will be distributed among 3-125 ml borosilicate glass bottles (PPR), 2-20 ml scintillation vials (pH, DO), 1-30 ml serum vial (DIC), 2-1000 ml amber HDPE bottles (BAC, TDR, CN, NUT, DOC, AN/CAT, CHL, PP, plus extra water to be used for additional experiments, in case of sample spillage, etc.), and 1-500 ml HDPE amber bottle (PHYTO). **Note**: Some samples are not collected at all depths (See Appendix 6.1).

Ideally, three people are required to perform a limno run; one person will take care of the pH, DO, and DIC samples; one person will operate the winch and handle the PPR and HDPE sample bottles, and the third person will distribute samples from the water sampler. To begin sampling, prepare the Niskin bottle for deployment, lower to specified depth and trigger the closure mechanism with a messenger. Bring the sampler up to the surface, drain ~10 ml, and gently invert 4x to thoroughly mix the sample. Place the Niskin bottle into the stand and attach the teflon tubing to the stopcock (always attach the same end of the tubing to the sampler to minimize contamination). Open the breather valve on the sampler and allow water to briefly flow into a bucket to rinse the tubing. When filling each sample bottle or vial it is important to insert the tubing to the bottom of the container and fill from the bottom up. Slowly remove the tubing, taking care not to disturb the sample. This procedure will reduce changes in gas concentrations inside the bottles. Because there is limited volume of sample, sparingly rinse the PPR, DIC, and HDPE bottles; the scintillation vials should be new (unopened flats).

- Firstly, fill the 3 PPR bottles (rinse 3X with sample water first) (overflow with ~50 ml of sample), replace the caps, and return samples to the PPR box (Never allow direct sunlight to shine on the bottles).
- Secondly, fill the DIC bottle (rinse 3X with sample water first) allowing the sample to displace 2X the volume (about 5 seconds). Immediately fix the sample, cap, and return to transport carrier.
- Thirdly, fill the pH and DO scintillation vials allowing the sample to displace 2X the volume of the vials (about 5 seconds). Immediately fix the DO sample and cap both samples, then return samples to the transport carrier.

- Finally, fill the HDPE bottles in the following order: 2 x 1000 ml amber (rinse 3X with sample water first), 500 ml amber (fill with 450 mls, leaving room for expansion). Once the HDPE bottles are filled, place them into a cooler. Continue on to the next sampling depth.

During the collection process it is important to monitor the time because the PPR samples should begin their incubation by 7:30 am. At Lake Hoare and Fryxell, typically all of the sample depths can be collected before inoculating the PPR samples with ^{14}C-bicarbonate and still begin the incubation period on time. However, at Lake Bonney it is best to collect all of the samples down to the last PPR depth, then inoculate the PPR samples, and continue the deep water sample collections after the PPR samples begin their incubation. Please consult Section 3.2 Primary Production for details on the inoculation procedure. While two people are preparing the PPR samples, the third person should prepare the incubation hole for deployment of samples. This includes removing ice from the hole, unwinding the PPR incubation line such that the samples may be readily attached and lowered into the water, and securing a tarpaulin over the hole with ice screws so the bottles may be handled in a darkened environment.

Once the samples have been collected and the PPR samples are incubating, begin the PAR time series logging (Section 5.2 PAR). It is important to record the time of day when the PPR incubation and PAR logging began, and the precise bottom depth sampled. For the return trip to camp make sure all of the samples are carefully packed in coolers for transport (avoid freezing) and the sampling hut is cleaned. Following the 24 h PPR incubation, remove the samples from the lake and place directly into the transport carrier. This procedure requires two people and must be performed under the tarpaulin. Finally, allow the PAR logging to complete a 24 h cycle before retrieving sensors.

Lake Ice Thickness and Piezometric Lake Depth

General Discussion
Lake ice thickness measurements are recorded at each hole drilled in the ice cap; this includes all sampling holes, the incubation hole, sediment trap holes and blue box holes. The "grid" system described in Appendix 6.8 to randomize measurements is no longer in use. Since then, statistical analyses by Dr. Priscu have shown that measurements from three ice holes are representative of the ice thickness measured by the grid system. Piezometric lake depth is measured at each sampling hole.

Materials
Tape measure
Aqua-Vu underwater viewing system
Messenger

Procedure
The Aqua-Vu underwater viewing system allows us to see the exact point where the bottom of the ice cover is located. Remove slush from the ice hole, and lower the Aqua-Vu underwater camera until the bottom of the ice cover is viewed through the above-water viewing system and centered vertically in the screen. Mark the distances from the bottom of the ice cover to the piezometric water level (z-water), and to the top of the ice cover (z-ice), on the viewing system cord and measure the distances with a tape measure once the viewing system is brought out of the ice hole. If the height of the ice surrounding the hole varies, make several measurements of z-ice and take an average. Alternately, a string can be held across the ice hole and z-ice measured to the string to obtain an average measurement. The piezometric water level is the level at which water rises up through the ice hole due to hydrostatic pressure caused by the permanent ice cover. Subtract z-water from z-ice to obtain freeboard (z-difference). Together these 3 measurements provide some insight into the topographical features of the ice cover as well as the density of the ice. Ice thickness measurements are performed on each hole drilled through the ice cover. Because most limnological sampling holes are drilled in low-lying areas, it was thought that ice thickness measurements were biased. Therefore, notes are taken to describe the topography of the ice surrounding the ice hole.

Piezometric lake depth (the distance from the piezometric water level in the ice hole to the bottom of the lake) is measured at each sampling hole at least one day before a limno run is performed. Lower the Aqua-Vu underwater camera until the bottom of the lake is visible through the above-water viewing system. Gently lower the camera until it touches the lake bottom and record the piezometric water level in the sampling hole. If the Aqua-Vu cord is not long enough to reach the bottom of the lake, attach a sample messenger to the end of a tape measure and lower the messenger to the lake bottom. Record the piezometric water level in the sampling hole. **Note**: Adjust the depth measurement to account for offset due to messenger.

Ablation measurements

Ablation measurements are taken by measuring the length of the LICOR cord that has ablated out of the ice at the blue boxes on each lake each season. These measurements are important for calibrating the depth change of the "blue box" LICOR sensors over time. During the 0607 season, a new buoy system for the PAR sensors was installed by Peter Doran's group (Figure 2). It is no longer necessary to measure ablation of the blue box LICOR cord each season.

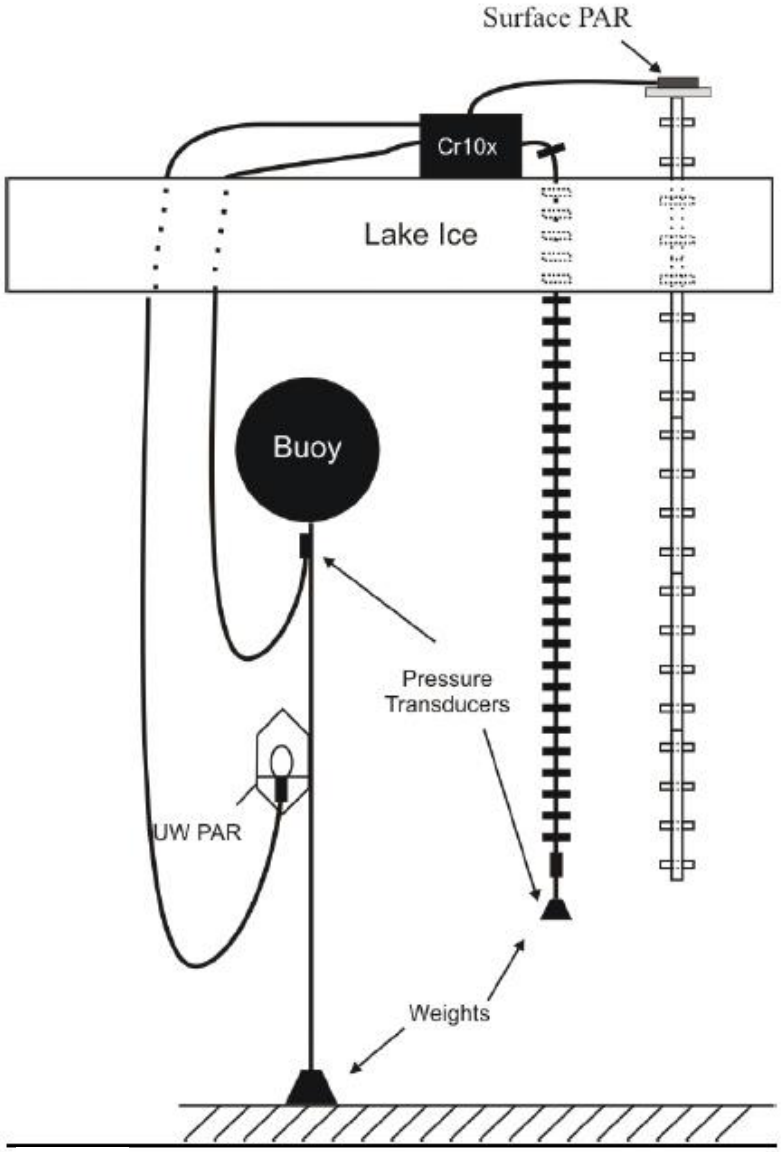


Fig. 2. Lake cross-section showing buoy, data logger, position of deployed sensors and ablation stake.

Pelagic Sediment Traps

Sediment Trap Location

The sediment traps in each lake have been located in the same area since 1993, and are marked with flags identifying their location. Each lake (traps were removed from Lake Fryxell and Lake Hoare in the late 1990's) contains two sediment trap arrays, containing three in-line traps (Table 1). The trap arrays are placed in the southern edge of the melted hole while the support rope is ran diagonally up to the northern edge of the hole. Therefore, in the following year, drill the hole near the rope (surface exit) and melt towards the south. Theoretically, the ice column directly above the sediment trap array contains little sediment, but undoubtedly there are small sediment pockets that will dump. Ideally, these sediment dumps will not directly enter the sediment traps.

Table 1. Depth of the inline sediment traps located on each array.

Trap Location	East Bonney	West Bonney	Hoare	Fryxell
	Sediment Trap Depth (m)			
Top	13	13	8	6
Middle	20	17	16	10
Bottom	35	35	29	18

Removal of Sediment Traps

Once the hole is melted and the array is free of the ice, slowly retrieve the sediment traps (this is a two person task). When the first trap is lifted out of the water, the second person grabs the rope below the trap and continues to haul up about 2 m of rope to provide some slack and ties off to the ice screw located near the hole. Meanwhile, the first person places the sediment trap upright into a bucket. Remove the honeycomb baffle with needle nose pliers. Any pieces of honeycomb that break off may be removed from the sample during sieving. While sitting on the chair, position the sediment trap between your legs and siphon off the water that remains inside the cone down to about 5 cm above the bottom of the cone. If the siphon freezes during this step use a 500 and 100 ml beaker to bail the water out of the cone, being careful not to create turbulence in the bottom of the trap. Place a 1000 ml HDPE bottle directly below the 125 ml HDPE bottle attached to the bottom of the sediment trap, and slowly unscrew the 125 ml bottle allowing it to fall directly into the 1000 ml bottle. Use a squirt bottle to rinse any sediment particles adhering to the sides of the trap into the 1000 ml bottle and cap. Pour the siphoned lake water into a gray water carboy. Repeat these steps until all traps in the array are removed. When the last trap is being removed, place the anchor into a second bucket to prevent benthic ooze from contaminating the surface area.

While the trap array is laying on the ice surface, measure the distance from the anchor to each trap and record. Also, measure the total ice thickness and piezometric water depth and calculate the depth at which each trap is located.

To deploy the array, have three 125 ml bottles with the correct salt:formalin solution ready to attach to each cone. Prepare the salt:formalin solution before removing the sediment traps. Add the appropriate mass of NaCl (Table 2) to 50 ml *DI* water containing 3 ml of formalin. Attach the appropriate bottle to the bottom trap (be careful not to cross thread the bottle to the trap) and begin to lower the anchor slowly into the water. When the trap reaches the water level, slowly fill the trap (~30 seconds); do not disturb the preservative in the 125 ml bottle. Continue this process with each trap and position the support rope properly when finished (see above).

Table 2. Mass of NaCl added to 50 ml *DI* water containing 3 ml formalin for each trap in each lake.

Trap Location	East Bonney	West Bonney	Hoare	Fryxell
		NaCl (g)		
Top	12	12	6	6
Middle	18	18	6	12
Bottom	18	18	6	12

Materials
1000 ml HDPE wide mouth bottles (1 trap^{-1})
125 ml HDPE wide mouth bottle (1 trap^{-1})
2 Buckets
Assorted Tri-pour plastic beakers (250, 400, 1000 ml)
Chair
DI rinse bottle
Elemental analyzer
Fiberglass sediment trap cones with honeycomb baffle (22 cm diameter)
Grey water carboy
Handwarmers
Ice screw
Needle nose pliers
Sieves with collection pan (250 and 62 μm mesh screen)
Siphon tubing

Reagents
Formalin (5%)
HCI (concentrated)
NaCL

Sediment Trap Processing
1. Preweigh the assorted plastic beakers and write the tare weight directly on the beaker. Also, record the tare weight in a lab book. For each sediment trap there will be three size fraction

categories (>250 μm, 250-62 μm, and <62 μm). The two larger size fractions usually fit into a 400 ml beaker and the <62 μm usually takes two 1000 ml beakers, because all of the rinse water is collected in the <62 μm size class.

2. Stack the sieves so that the 250 μm is on top, the 62 μm is in the middle and the collection pan is on the bottom. Place the entire sediment sample into the top sieve and pour ~ 1000 ml of *DI* water onto the sample and begin to shake the sieves vigorously. Ideally the water should be of similar NaCl concentration as the material in the trap. Because all of the water is collected, it is best to use as little water as possible to sieve the sample. Also, it is helpful to have a second collection pan to place under the sieves so that once all of the water has passed through the sieves, the bottom pan may be removed to pass the water through the sample again. This procedure must be repeated at least 25 times or until each sample is completely separated into three size classes.

3. Once the sediment is separated into three size classes, use a rinse bottle to transfer the sediment into a labeled beaker (lake, depth, size class, and tare weight)

4. Dry all size fractions at 105 °C for > 24 h.

5. Weigh each beaker and record the total weight. Obtain a dry weight of each fraction by subtracting the tare weight from the total weight.

6. Analyze a known amount of each size fraction for particulate carbon and nitrogen using an elemental analyzer. **Refer to the Carbon and Nitrogen analysis of filters and sediment samples (Elemental Analyzer) section for a complete description of sediment analysis.**

Chlorophyll-*a* Extraction (revised October 2008)

General Discussion

This procedure describes the extraction of chlorophyll-a from glass fiber filters using 90% acetone from a known volume of filtered water, and the ensuing analysis of the extracted chlorophyll-a using fluorescence. The method is essentially that described by Holm-Hansen et al. (1965) as modified by Welschemeyer (1994) to reduce interference by chlorophyll-b and phaeopigments. The Welschemeyer method provides sensitive measurements of extracted chlorophyll-a free from the errors associated with conventional acidification techniques. Fluorometric optical configurations are optimized to produce maximum sensitivity to chlorophyll-a while maintaining desensitized responses from both chlorophyll-b and pheopigments. This method requires a single fluorescence determination and provides adequate sensitivity for small sample sizes (<200 ml) even in the most oligotrophic marine and freshwater environments. Note that the Welschemeyer method incorporates cross-comparison to a standard calibrated with a spectrophotometer. Samples containing high levels of chlorophyll-a (e.g., mat samples) should be analyzed directly on a spectrophotometer to avoid dilution problems and interference from accessory pigments.

A description of how to make your working standards is provided along with specific details in obtaining consistent standard curves. The filtration step has been adapted to allow the collection of filtrate for nutrient and DOC analyses**. Importantly, chlorophyll-a is very sensitive to low pH and high light (and to some extent, high temperature). Hence, all sample collection, filtration, extraction, and fluorometric readings should be conducted in a neutral (or high) pH environment and reduced light. Never work in an environment where acid containers exist (e.g., where carboys of acid exist, where glassware is being rinsed with acid) and maintain all samples (and standards) under low light from the time of collection to final analysis.**

The fluorometer should be calibrated before extraction of lake samples to ensure that the fluorometer and spectrophometer are working properly.

Materials

Sampling Site

- 1000-mL Amber HDPE bottles (pre-labeled with the water depth that will be sampled). This bottle should NOT be acid rinsed to avoid degradation of chlorophyll-a, which is very sensitive to acidic conditions and high light.
- Cooler (Do Not Freeze)

Dry Valleys Lab (filtering lab)

- Vacuum pump (set to low vacuum pressure <7 inches Hg)
- 100-mL graduated cylinder (plastic)

- Bell jar filtering apparatus with bottle stands (to collect filtrate for other analyses)
- 25-mm x 200-mL polysulfone filter funnels
- 25-mm GF/F filters, pre-combusted and acidified. (Combustion protocol: combust at 475^{o}C for 4 hrs spread out on aluminum foil that will serve as a wrapper after combustion and acidification. Acidification protocol: soak in 1% HCL for 3-4 hours, rinse 4X with DIW, bring the pH to 8-9 with NaOH, rinse 2X with DIW, lay out on combusted foil to dry, and wrap in foil once dried). Combusted and acidified filters are only necessary if certain filtrate samples are being collected – they are not required for Chlorophyll analysis. Since combusted filters are required for Dissolved Organic Carbon (DOC) filtrate (collected from one Chlorophyll replicate), and acidified filters should be used for nutrient (Soluble Reactive Phosphorus) filtrate (collected from one Chlorophyll replicate), it is recommended to combust and acidify chlorophyll-a filters.
- 3 Forceps (one for moving dry filters; two for removing filters after filtration)
- Glassine envelopes (used to store sample filters after filtration)
- Aluminum Foil (used to wrap glassine envelopes containing filters for storage at 4°C)
- Wash bottle (for DI water)
- 125-mL narrow mouth HDPE bottle (acid washed) – for collection of filtrate for Nutrient analysis
- 125-mL amber borosilicate glass bottle (acid washed & combusted) – for collection of filtrate for DOC analysis

<u>MCM Crary Lab - Labware</u> (NOTE: this glassware is stored in a labeled box in the Crary Lab and should be returned to this box when not in use!)

- 10 ml automatic dispenser
- Glass scintillation vials (20-ml with HDPE or foil-lined caps)
- Glass cuvettes (13x100 mm) for use in fluorometer
- 4 ml Pipettor (e.g. P5000 Pipetteman with 5 ml pipette tips)
- 1 cm glass cuvette for spectrophotometer (Perkin Elmer LS-50B (Stock # 050271, Crary # D10913) or equivalent)
- 3 ea – 100 ml Pyrex volumetric flasks (type A)
- 5 ea – 25 ml Pyrex volumetric flasks (type A)
- 7 ea – 10 ml Pyrex volumetric flasks (type A)
- 1 ea – 2000 ml Pyrex graduated cylinder (type TD)
- 2 ea – 10 ml glass volumetric pipettes (type A)
- 4 ea – 5 ml glass volumetric pipettes (type A)
- 2 ea – 3 ml glass volumetric pipettes (type A)
- 1 ea – 2 ml glass volumetric pipette (type A)
- 3 ea – 1 ml glass volumetric pipette (type A)
- 2 ea - 0.5 ml glass volumetric pipette (type A)
- Pasteur pipette or eye dropper (for acidifying with 3N HCL)
- Pipette Pump for use with the glass volumetric pipettes
- 125 ml amber bottle (Used to store concentrate)
- 2 ea – wash bottles (For 90% Acetone)

- 4 funnels (HDPE or Glass)
- 500 ml beaker (waste beaker)
- 4 L Acetone bottles (Once a bottle of Acetone is empty, the 4 L amber bottle is used to keep your mixed reagents and waste. **Make Sure They Are Well Labeled.**)

- **Note about cleaning glassware.** Use a lab detergent that is basic (high pH, e.g. Liquinox) to
- clean all glassware. After washing with detergent, rinse the glassware 6 times with tap water.
- Then rinse 3 times with DI water to remove the tap water. Before using the glassware, rinse with
- 90% acetone. Glass pipettes should also be washed following this method.

MCM Crary Lab - Reagents

- Chlorophyll-a standard, 1 mg from *Anacystis nidulans* (Sigma, C6144-1MG, 066K1862). Other purified sources can be used (e.g., Spinach standard from SIGMA), but *A. nidulans* is preferred because this organism contains no chlorophyll-b.
- 90% Acetone: Acetone (e.g. Baker HPLC grade, 632262, 9002-03). Pour 200 ml of DI water into a 2000 ml graduated cylinder (rinse with 90% acetone prior to use) and bring to volume (2000 ml) with 100% acetone. Place parafilm on the top of the graduated cylinder and invert the solution 20 times. Once this is complete, label a 4L empty "Acetone bottle" (or other clean amber bottle): 90% Acetone, date, and your initials. Pour new reagent into the bottle carefully.

Notes: Wear gloves at all times. Acetone should be HPLC grade and this procedure should be completed in an acid free hood. Do not forget to rinse the glassware with 90% acetone before starting. Make sure you pull the funnel out of the volumetric flask when checking the volume.

- 3N Hydrochloric Acid (add 25 ml of concentrated HCl (12N) to 75 ml DIW). Store in a 125 ml Nalgene (HDPE) bottle on the shelf.

MCM Crary Lab-- Instruments

- Fluorometer (Turner 10-AU-10) configured for Chlorophyll-a analysis as described by Welschmeyer (1994):

  - Lamp: "Blue" F4T4.5B2 (F4T4½B2); Turner #10-089. It should have an "A" imprinted on the metal end and is often referred to as: "blue, custom color, Sequoia Turner". Alternatively, a blue lamp (type 9005)-Turner Designs (No. 10-089) can be used which has identical spectral characteristics. A F4T4D daylight lamp, can provide similar selectivity but with about a 2-fold reduction in sensitivity.
  - Excitation Filter (blue): 436BP10 047 9401; Turner #10-113
  - Emission Filter (red): 680BP10 357 9405; Turner #10-115

  NOTE: (The excitation and emission filters should have mirrored side toward the actinic light (ie, facing the direction of incoming light))

- Sample holder: for 13-cm long tubes

- Visible-range Spectrophotometer (e.g. Beckman DU-640 or equivalent) with:
  - spectral bandwidth <2 nm
- 1 cm glass cuvette for spectrophotometer (Perkin Elmer LS-50B (Stock # 050271, Crary # D10913) or equivalent)

NOTE: A dual beam spectrophotometer is preferred. Use 90% acetone in the reference cuvette.

Procedures

A. Sample Collection

Fill the 1000ml Amber HDPE bottle (rinse 3x with sample water before filling with sample) with sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Each bottle should be labeled with: location, date, depth, and limno run. Place bottles in a cooler for transport – avoid freezing.

STORAGE: These bottles should be stored in the dark at ca. 4^{o}C—DO NOT FREEZE!

B. Filtration -- Dry Valleys Filtering Lab

- **Notes about Lighting and Acids**: Chlorophyll-a measurements must be completed in a darkened, but not dark, environment (NO DIRECT SUNLIGHT). All light in the room should be indirect or diffused, so do not use a flashlight or headlamp to shine a bright beam on the sample ("red" headlamp diodes emit light at a wavelength, 660 nm, which is highly efficient at exciting chlorophyll molecules, so do not shine directly on sample). Use a light meter to determine appropriate amount of light. Keep light below 1 μE m^{-2} s^{-1} (ca. 5 footcandles, or 50 lux). WORK WELL AWAY FROM ACIDS AND ACID FUMES!

1. Chlorophyll-a samples are taken from the 1000 ml amber HDPE bottle. Gently invert the bottle 3x, thoroughly mixing sample, and decant 100 ml into a 100 ml graduated cylinder (avoid using larger graduated cylinders – accuracy and precision will be lower). Some depths at ELB and WLB require more sample to be filtered because of low chl-a concentration (Table 3): **ELB 22-37m = 200 ml; WLB 17-38m = 200 ml. Also, Miers = 200 ml.**

Table 3. Volume of lake water filtered for chl analysis.

Lake	ml Lake Water Filtered
Fryxell	100 ml
Hoare	100 ml
Miers	200 ml
East Lobe Bonney	4.5-20m – 100 ml; 22-37m = 200 ml
West Lobe Bonney	4.5-15m – 100 ml; 17-38m = 200 ml

♦♦♦♦♦ Record the volume of water filtered for each depth in each lake. ♦♦♦♦♦

2. Place a combusted and acidified 25 mm GF/F filter onto the filter base which is mounted on a vacuum bell jar and replace tower. To collect filtrate, place either an acid washed 125 ml HDPE bottle (for nutrient samples) or acid washed and combusted 125 ml amber borosilicate glass bottle (for dissolved organic carbon samples) under the filter base—MAKE SURE THAT THE ACID WASHED BOTTLES HAVE BEEN RINSED WELL WITH DIW BEFORE USE.

3. Once the GF/F filter is in place and the tower has been tightened onto the filter base, pour the 100ml of sample from the graduated cylinder into the tower. Filter the sample under low pressure (<7 inches Hg). Do not rinse the filter tower with DI water. Once the sample has been completely filtered, carefully remove the tower from the filter base. With two forceps, carefully remove the filter and fold it in half (organic matter inside), and carefully place the folded filter into a glassine envelope labeled with: <Chl-a>, <lake>, <depth>, and <date>. Wrap glassine envelopes containing filters in aluminum foil to keep them in the dark (glassine envelopes can be stacked and wrapped in foil together). Remove the bell jar and cap the filtrate bottle. Properly store both filter and filtrate samples until further analysis. Filters can be kept on ice in the lab (in a beaker (to keep them dry) placed in an ice bucket) during the filtration process, and then placed in a freezer (-20 C) until transport to the Crary Laboratory. DOC and Nutrient samples can be kept on the floor of the lab during the filtration process, then placed at 4^{o}C and -20^{o}C, respectively, until transport to the Crary Laboratory.

4. Repeat steps 1-3 above for a replicate chlorophyll-a sample and either a nutrient or DOC sample. Replicate filters can be placed in the same glassine envelope, but make sure that they are not touching each other, or they will freeze together and be difficult to separate for the extraction process. Rinse the filter tower with DI water after both replicate samples are filtered.

5. Once all samples are filtered, label the foil pouch containing the glassine envelopes with <Chl-a>, <lake>, and <date> and immediately freeze until the extraction procedure.

STORAGE: Nutrients and Chl-a filters are stored frozen in the dark at -20^{o}C; DOC samples are **Do Not Freeze** and should be stored at 4^{o}C. Chl-a samples should not be left on lab bench for extended periods of time - keep them frozen and dark!

C. Preparing Chlorophyll standards

***Chlorophyll-a Stock Concentrate* ~10,000 µg/L:**

Notes about Lighting and Acids: All chlorophyll-a stocks should be prepared and handled in a low light environment. All light in the room should be indirect or diffused, so do not use a flashlight or headlamp to shine a bright beam on the sample ("red" headlamp diodes emit light at a wavelength, 660 nm, which is highly efficient at exciting chlorophyll molecules – so do not shine directly on sample). Use a light meter to determine appropriate amount of light. Keep light below 1 µmol photons m^{-2} s^{-1} (ca. 5 footcandles, or 50 lux). KEEP THIS STOCK AWAY FROM ACIDS OR ACID FUMES!

Tap the glass ampoule containing the chlorophyll-a standard (1 mg *Anacystis nidulans)* to move all of the powder to the bottom. Using a paper towel, carefully break the top of the ampoule and keep both pieces. Carefully tip the bottom portion of the ampoule into a 100 ml glass volumetric flask and flick the ampoule to get the powder to drop into the flask. Once most of the powder has been removed, fill the top and bottom of the ampoule with 90 % acetone, and pipette the liquid out of the ampoule to remove any remaining chlorophyll using a 100 µl pipette. Repeat filling the ampoule top and bottom until the liquid is not green. Dispose of both pieces of the broken ampoule properly. Bring the chlorophyll-a concentrate up to 100 ml in the volumetric flask with 90% acetone. Pour this concentrate into a labeled125 ml glass amber bottle wrapped in aluminum foil for storage. The bottle should be kept in a dark refrigerator at 4 ^{o}C (with NO ACIDS). This concentrated stock can be used for the entire season to make Diluted Stocks "A" and "B," and the working standards.

Chlorophyll-a Dilute Stock A ~ 1,000 µgChl-a/l
Using a 10 ml glass volumetric pipette, remove 10 ml of the 10,000 µg/l Chlorophyll-a Stock Concentrate from the 125 ml amber bottle and place it into a 100 ml glass volumetric flask. Bring the solution up to 100 ml volume with 90% acetone, invert 20x, wrap in aluminum foil and label "Dilute Stock A ~1000 µgChl-a/l". Stock A should always be made fresh before making Dilute Stock B and new working standards (see below). Store in the dark at 4 ^{o}C.

Chlorophyll-a Dilute Stock B ~ 100 µgChl-a/l
Using a 10 ml glass volumetric pipette, remove 10 ml of Chlorophyll-a Stock A from the 100 ml volumetric flask and place it in a new 100 ml volumetric flask. Bring solution up to 100 ml volume with 90% acetone, invert 20x, wrap in aluminum foil and label "Dilute Stock B ~100 µgChl-a/l". Stock B should always be made fresh before making new working standards. Store in the dark at 4 ^{o}C.

Standardizing the Chlorophyll-a Stock Concentrate and Diluted Stocks
The Chlorophyll-a Stock solutions must be measured on a spectrophotometer to determine the *actual* concentration. (Typically the stock concentrate is the only standard that is calibrated on the spectrophometer. Because this is a relatively easy measurement, we measure the concentrations in Dilute stocks "A" and "B" to ensure that dilutions were made properly and no degradation occurred during the dilution step). The dilute stocks are then diluted further and used to calibrate the fluorometer as described below.

Turn on the Beckman DU-640 Spectrophotometer (or equivalent), click UV ON and VIS ON and allow it to warm up for >30 minutes (Figure 1). Click FIXED WAVELENGTH (Figure 1).

Figure1. Beckman DU-640 Spectrophotometer screen.

Click “Method” (Figure 2).

Figure 2. Beckman DU-640 Spectrophotometer screen.

Choose the ChlA method saved in the computer "A:\chlA." This method is set to run samples at 665 and 750 nm wavelengths (Figure 3). Fill the 1 cm cuvette with 90% acetone, wipe off the sides with a Kimwipe, and use to zero the instrument (click BLANK (Figure 3)) at 665 nm and 750 nm (the 90% acetone blank readings should be very similar. If not, zero the instrument at 750 nm and write down the value at 665 nm). The blank sample can be read at 665 and 750 nm by clicking "Read Samples" (Figure 3). Note that the 1st cell read is the cell towards the back of the instrument.

Figure 3. Beckman DU-640 Spectrophotometer screen.

Tap the cuvette on a Kimwipe to remove the acetone, replace the 90% acetone blank with the Chlorophyll-a Stock Concentrate, and measure absorbance at 665 nm and 750 nm (non-acidified readings are denoted by a subscript "o") by clicking "Read Samples" (Figure 3). Remove the cuvette and acidify the Concentrate in the cuvette by adding 2 drops (from a glass Pasteur pipette or eye dropper) of 3N HCl. Mix by placing parafilm over the cuvette and inverting 6x. Wipe the sides of the cuvette with a Kimwipe and return to the cuvette holder in the spectrophotometer in the exact orientation from which it was removed. Reread absorbances at 665nm and 750 nm (acidified readings are denoted by a subscript "a"). The acidification step will correct for possible phaeophytin in the sample. NOTE: The absorbance at 750 nm is used to correct for light scatter and potential non-pigment absorbance of solvent, particles, etc. Repeat the above procedure for the Chlorophyll-a Stock Concentrate to obtain three replicates, then repeat three times each for Diluted Stocks A and B. The cuvette should be rinsed 6x with DIW and 3x with 90% acetone between each sample. The following table (Table 4) should be entered in the "chlorophyll-a" data book for the "concentrated" and

"diluted" (i.e., "A" and "B") standard solutions. The date, name of the analyst and samples processed using standard curves made with these solutions should also be entered.

Table 4. Table for spectrophotometer readings of concentrated and diluted standard solutions.

	Wavelength (nm)				Chl-a concn	Avg Chl-a concn
	665_o	750_o	665_a	750_a	(µg/l)	(µg/l)
Concentrate rep 1						
Concentrate rep 2						
Concentrate rep 3						
Dilute A Stock rep 1						
Dilute A Stock rep 2						
Dilute A Stock rep 3						
Dilute B Stock rep 1						
Dilute B Stock rep 2						
Dilute B Stock rep 3						
Date prepared:						
Analyst:						
Samples processed:						

Determine the chlorophyll-a content of each stock using the following equation (Strickland and Parsons 1972; Parsons et al. 1984):

$$\text{Chl-a } (\mu g/l) = [26.7*((ABS665_o - ABS665_a) - (ABS750_o - ABS\ 750_a))*1000]/l$$

Where:

- $ABS665_o$ = ABS at 665 nm with no acid
- $ABS665_a$ = ABS at 665 nm plus acid
- $ABS750_o$ = ABS at 750 nm with no acid
- $ABS750_a$ = ABS at 750 nm with acid
- l = cuvette path length (1 cm)
- 26.7 is a value that combines the extinction coefficient for pure chlorophyll-a in 90% acetone (89 L g^{-1} cm^{-1}) in concert with an acidification factor that represents the absorbance ratio of pure chlorophyll-a:phaeophytin-a following acidification. Specifically, 26.7 was derived as follows: [((1/89 L g^{-1} cm^{-1})*(1000L m^{-3}))]=11.23g*cm/m^3. Hence, 11.23g*cm/m^3 * 2.38 (the acidification ratio)=26.7 g*cm/m^3.

Standardizing the Fluorometer

- The stock chlorophyll-a solutions prepared above will be used to prepare working standard dilutions of chlorophyll-*a* using 90% acetone to dilute the standards. (Use volumetric

glassware to make these standard dilutions). The working standards will then be used to calibrate the fluorometer. Note that the concentration of the working standards is based on the spectrophotometrically determined concentration of the Stock Concentrate, which should be ~10,000 µChl-a/l, and the Diluted Stocks "A" and "B" which should represent 10 and 100-fold dilutions of the concentrated stock. Therefore, the actual concentrations of the working standards will need to be computed from the spectrophotometrically determined concentration of the stocks. This calibration should be done monthly from the stored Concentrated Stock (the concentration of the Concentrated Stock should be determined using the spectrophotometer as described above before each calibration).

Working Standards

Notes: All volumetric flasks should be pre-labeled with numbers indicating the amount (ml) of Dilute Stock A and Dilute Stock B you should pipette in each flask. Dilute A is indicated by red tape and Dilute B is indicated by white. While bringing the standards up to volume, if any are over filled, start over. Make sure you do not have any air bubbles. After using the glass pipettes rinse them with 90% acetone 5 times before placing them back in the aluminum foil. Make sure to invert all flasks 20x to ensure complete mixing. Make a table as outlined below (Table 5) in the chlorophyll-a data book. These standards should be prepared before the samples are extracted to ensure that the fluorometer is responding appropriately (comparison to regression parameters of old standard curves should yield similar parameters (slope and intercept).

Table 5. Volume of 90% acetone and stock solution used to make working standards.

Stock	**Stock concentrations (*µ*g/l)**	**Volumetric Pipette (ml): [Volume of stock solution]**	**Volumetric Flask (ml): [Bring stock solution to mark with 90% acetone]**	**Chl-a (*µ*g/l) [compute exact values from spectrophotometrically determined stock concentrations]**
Stock Concentrate ~10,000 µg/l	Determined by spectrophotometer			
Dilute A ~1,000 µg/l	Determined by spectrophotometer	5	10	~500

Dilute A ~1,000 µg/l	Determined by spectrophotometer	3	10	~300
Dilute A ~1,000 µg/l	Determined by spectrophotometer	5	25	~200
Dilute A ~1,000 µg/l	Determined by spectrophotometer	1	10	~100
Dilute A ~1,000 µg/l	Determined by spectrophotometer	2	25	~80
Dilute A ~1,000 µg/l	Determined by spectrophotometer	1	25	~40
Dilute B ~100 µg/l	Determined by spectrophotometer	5	10	~50
Dilute B ~100 µg/l	Determined by spectrophotometer	3	10	~30
Dilute B ~100 µg/l	Determined by spectrophotometer	5	25	~20
Dilute B ~100 µg/l	Determined by spectrophotometer	1	10	~10
Dilute B ~100 µg/l	Determined by spectrophotometer	0.5	10	~5
Dilute B ~100 µg/l	Determined by spectrophotometer	0.5	25	~2
Date prepared:				
Analyst:				
Samples processed:				

Read each of these working standards on the fluorometer according to the following protocol:

1. Turn on Fluorometer and allow to warm up for at least 30 minutes.

2. Dispense ca. 4 ml of each working standard into a 13-mm x 100 mm glass cuvette. Wipe cuvette exterior with Kimwipe to remove all liquids and place cuvette into the fluorometer sample chamber.

3. Allow fluorometer to autoscale, then take reading and record values in notebook.

4. Periodically (e.g. every 10 standards) read a blank sample consisting of 90% acetone to check instrument baseline.

5. Rinse cuvettes three times with DI water and three times with 90% acetone to clean them between standards, and tap on Kimwipe to remove remaining liquid.

6. Prepare a standard curve of chlorophyll-a concentration vs fluorescence. A typical curve prepared during the 2008 season on 11 April follows (Table 6; Figure 3). Slopes and intercepts should be similar from run to run (and year to year) if all procedures are followed properly and the fluorometer is not altered by such things as changes in gain or lamp problems (the lamp should be changed every other year). Always run the 0.8 and 4 solid standards to check for fluorometer drift over time.

Table 6. A typical standard curve of chlorophyll-a concentration vs fluorescence prepared during the 2008 season.

vol Std "A" (ml)	vol Std "B" (ml)	total vol (ml)	µgChl-a/l	Fluorescence
Solid 0.8				0.694
Solid 4				3.650
BLK (90% acetone)	0	0	0	0.029
0	0.5	25	1.9	0.209
0	0.5	10	4.6	0.482
0	1	10	9.3	0.945
0	2	10	18.6	1.950
0	3	10	27.9	2.940
1	0	25	37.2	3.970
0	5	10	46.5	4.870
2	0	25	74.4	7.970
1	0	10	92.9	10.100
2	0	10	185.9	19.500
3	0	10	278.8	28.700
5	0	10	464.7	46.400

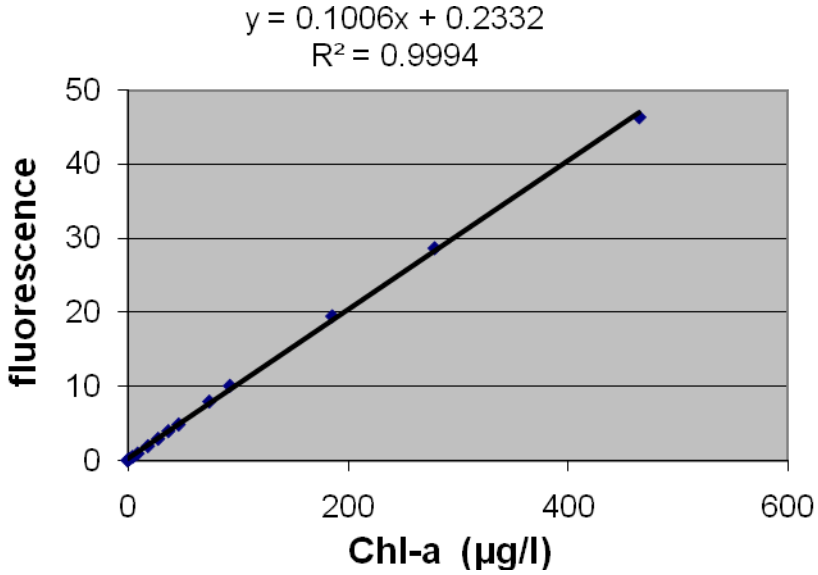


Figure 3. A typical standard curve of chlorophyll-a concentration vs fluorescence prepared during the 2008 season.

The concentration of chlorophyll-a in the sample extract is calculated from the standard curve. The final chlorophyll-a in the lakewater is calculated as follows:

Chlorophyll-a (µg/l) = (F_o – y-intercept/slope) * (ml extracted/ml filtered)

Where:

F_o = measured sample fluorescence
y-intercept = fluorescence when Chl-a concentration is zero
slope = fluorescence/Chl-a (µg/l)
ml extractred = ml of 90% acetone used to extract the Chl-a on the filters

- ml filtered = ml of actual sample filtered in the field

Method Detection Limit

The method detection limit is calculated by running the lowest standard seven times to determine F_o and F_a, and using the response values from these replicates to solve for chl-*a* using the standard curve equation. The standard deviation from these seven chl-*a* values is multiplied by 3.15 to give the method detection limit.

D. Chlorophyll Extraction and Measurement -- MCM Crary Lab

Notes about Lighting and Acids: Chlorophyll-a measurements must be completed in a darkened, but not dark, environment. All light in the room should be indirect or diffused, so do not use a flashlight or headlamp to shine a bright beam on the sample ("red" headlamp

diodes emit light at a wavelength, 660 nm, which is highly efficient at exciting chlorophyll molecules, so do not shine directly on sample). Use a light meter to determine appropriate amount of light. Keep light below 1 $\mu E\ m^{-2}\ s^{-1}$ (ca. 5 footcandles, or 50 lux). WORK WELL AWAY FROM ACIDS AND ACID FUMES! Use the dark room in phase 2 of Crary if possible.

1. Place each filter into a labeled 20 ml scintillation vial.

2. Dispense 10 ml of solvent (90% acetone) into each vial using an automatic 10 ml pump dispenser and vortex, making sure the filter remains in the 90% acetone following vortexing.

3. Incubate the samples for ~24 hours in the dark at 4 ^{o}C, vortexing the samples (making sure the filter remains in the 90% acetone following vortexing) gently for 15 sec each near the middle of the extraction.

4. After extraction, vortex for 15 seconds (making sure the filter remains in the 90% acetone following vortexing), and allow to settle in the dark at 4 ^{o}C for 1 hour (keep cold while running samples?).

5. Turn on Fluorometer and allow to warm up for at least 30 minutes.

6. Run the solid standards and a blank (reading of solid standards should remain consistent).

7. Dispense ca. 4 ml of extract into a 13-mm x 100 mm glass cuvette. Wipe cuvette exterior with Kimwipe to remove all liquids and place cuvette into the fluorometer sample chamber.

8. Allow fluorometer to autoscale, then take reading and record values in notebook. Be sure to check that sample responses fall within the range of the standard curve.

9. Periodically (e.g. every 10 samples) read a blank sample consisting of 90% acetone to check instrument baseline.

10. Rinse cuvettes three times with DI water and three times with 90% acetone to clean them between samples, and tap on Kimwipe to remove remaining liquid.

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Phytoplankton Enumeration and Biomass

General Discussion

Phytoplankton enumeration and biomass estimates are performed on Lugol's preserved samples. A modified Utermöhl method is used to identify and enumerate phytoplankton with an inverted microscope. Consistent taxonomy is the most important consideration when counting and identifying phytoplankton. In order to compare phytoplankton taxa from one year to the next, "type" specimens must be available. The MCM LTER maintains a photographic archive along with archived samples for the comparison and identification of phytoplankton.

Materials

Sampling Hut

500 ml Amber HDPE bottles

Dry Valleys Lab

Eppendorf repeater pipet and tips

Off the Ice

Algal references (http://huey.colorado.edu/LTER/lakedata.html)

Inverted microscope with high quality oil immersion lens and high numerical aperture (NA), equipped with phase contrast lenses or differential interference contrast (DIC), camera attachment, camera, film (Nikon Diophot)

Ocular micrometer (for measurement of cell dimensions)

Settling chambers and removable settling columns (10 ml)

Stage micrometer with 1 μm graduations (for calibration of ocular micrometer, Whipple grid and field of view)

Reagents

Dry Valleys Lab

Lugol's Solution: Dissolve 20 g potassium iodide (KI) and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid (made in Crary Lab and transported to Dry Valleys for addition to samples).

Procedure

Sample Collection

1. Fill a 500 ml HDPE amber bottle with 450 ml of sample (leaving room for expansion) from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport. Note that not all depths (m) are collected for all lakes for phytoplankton enumeration and biomass (Table 7):

Table 7. Depths (m) collected for phytoplankton enumeration and biomass.

Fryxell (m)	Hoare (m)	Miers (m)	ELB (m)	WLB (m)
4.5	4.5	5	4.5	4.5
5	5	7	6	5

6	8	9	10	10
8	12	11	13	13
9	16	13	18	20
10	20	15	30	
12	25	16		
15		17		
		18		
		19		

Below step to be conducted in Dry Valleys Lab

2. Immediately preserve samples with 5 ml Lugol's solution using Eppendorf repeater pipet. Close the lids tightly, seal them with electrical tape and store samples at 4°C (Do not freeze) in the dark until transport to MCM (*All samples are sent to Diane McKnight @ University of Colorado for analysis: INSTAAR).*

Below steps to be conducted off the ice

3. Gently invert the HDPE bottle to thoroughly mix the sample; decant 100 ml of sample into a 100 ml graduated cylinder. Cover the opening of the cylinder with parafilm.

4. Allow the 100 ml sample to settle for 5 days. A standard method for settling times is 4 hours per cm of water column height (i.e. a graduated cylinder containing a sample height of 30 cm should settle for 120 hours; J. Priscu personal communication). After settling, siphon off 82 ml of sample with a "J" shaped Pasteur pipet. To construct a siphon; heat the tip of the Pasteur pipet with an open flame and carefully bend the tip into a "J" shape. Be careful not to heat the tip too much, otherwise the glass will melt. Attach the pipet tip to a filter flask and vacuum pump. Hold the "J" shaped tip just below the surface level and gently siphon the upper 82 ml of water. **Note:** The volume of water siphoned will depend on the volume of the Utermöhl chamber being used (i.e., the actual volume of the 10 ml Utermöhl chamber the Priscu lab uses is 18 ml).

5. Gently swirl the remaining 18 ml sample and transfer to a 10 ml Utermöhl chamber. Allow the Utermöhl sample to settle for another 20 hours (4 hours per cm of sample height) before preparing slide.

6. Prepare the slide by slipping off the Utermöhl tower, and immediately count the sample using an inverted microscope.

7. At least 100 individuals of the most numerous algae are counted per sample at 400× magnification with species identification being determined at 1000×. To determine the number of cells of any particular species per unit volume (cells ml^{-1}) use the following equation:

$$\text{Algal cells ml}^{-1} = \frac{C \times A_U}{F \times A_f \times V}$$

where C is the total number of algal cells counted, A_U is the area of the Utermöhl slide mount, F is the total number of fields counted, A_f is the area of the field, and V is the volume of sample settled.

The total number of individuals counted is dependent on the number of taxa, but usually ranges between 300 and 500. The number of individuals counted is variable; the investigator should adjust the number of cells counted in order to obtain an acceptable counting error (Table 8).

Table 8. Accuracy obtained at 0.95 confidence limits at differing size counts (Lund et al., 1958)

	Approximate 0.95 confidence limits	
Number of Organisms	As percentage of count	Range
4	±100 %	0-8
16	±50 %	8-24
100	±20 %	80-120
400	±10 %	360-440
1600	±5 %	1,520-1,680
10,000	±2 %	9,800-10,200
40,000	±1 %	39,600-40,400

If the distribution of organisms is random and fits a Poisson curve, the counting error (based on 95 % confidence limits) may be estimated using the following equation:

$$\text{Counting error} = \frac{2}{\sqrt{N}}(100\%)$$

where N is the number of algal cells counted. Counting errors in past analyses have ranged between 13 and 26%, and vary depending on species. While some species tend to be evenly distributed within the water column, others form colonies, or localized distributions. These biological realities will greatly affect the estimates obtained in a count. Algal species identification are made using Geitler (1932), Seaburg et al. (1979) and Prescott (1962).

8. Cell volumes are estimated for dominant taxa by measuring cell dimensions (length, width) of 10 individuals and using closest goemetric formulas of Willen (1976) and Tikkanen (1986). Because algal cell volumes may change over time, due to seasonal changes in environment, it is necessary to calculate cell volumes based on samples procured throughout

the season. For rare taxa, it is not possible to make as many measurements, and volume estimates must be made from fewer cell measurements.

9. Once the sample is counted, transfer the sample to a 10 ml glass vial (with a Teflon cap) and fill the vial with the sample that remained in the Utermöhl tower. Add 100 µl of Lugol's and archive the sample and store at 4°C in the dark. **Note:** Make sure the sample is labeled properly (i.e. Location code, date and depth).

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Phytoplankton Primary Production (PPR) Determined by ^{14}C Incorporation

General Discussion

Primary production is the centralized theme common to all LTER projects, especially projects where aquatic environments are the main focus. The McMurdo Dry Valley lakes represent the most productive systems in the ice-free regions of Antarctica. However, due to the extreme supersaturation of gases in these systems, estimates of primary production are very difficult to quantify measuring changes in O_2 evolution and CO_2 consumption. Therefore, we measure primary productivity using an *in-situ* $^{14}CO_2$ uptake method. A known amount of radiolabeled bicarbonate is added to a sample containing a known amount of dissolved inorganic carbon (CO_2) and the samples are allowed to incubate at their respective depth, in the lake. Following *in-situ* photosynthesis, samples are filtered, treated, and analyzed for their amount of radioactivity incorporated. Differential uptake of radiolabeled carbon is corrected for and estimates of primary productivity ($\mu g\ C\ l^{-1}\ d^{-1}$) are obtained for specific layers of the lake through the photic zone.

Materials

Sampling Hut

125 ml borosilicate glass bottles with polyethylene-lined screw caps (3 bottles per depth, 2-light and 1-dark)
P1000 Gilson Pipetman and tips (RAD ONLY)
List of isotope addition volumes
PPR bottle transport carrier
Radioisotope safety tray (lined with Benchcoat)
Vinyl gloves (at least 2 pair per person)
Ziplock for solid waste
Depth calibrated incubation line and clips

Dry Valleys Lab

25 mm GF/F filters
Filter manifold with 25 mm Polysulfone filter towers
Vacuum pump
Eppendorf repeater pipet with tips for HCL (NO RADS)
Filter forceps
Glass scintillation vials (20 ml with HDPE cone caps)
Heating block
Benchcoat
Kimwipes
Rad waste containers

MCM Crary Lab

Repipettor

Reagents

Sampling Hut

Ampulated ^{14}C carbonate/bicarbonate: (Activity should be between 100-120 $\mu Ci\ ml^{-1}$, pH ~9.5)

Dry Valleys Lab
3 N HCl
MCM Crary Lab
Cytoscint ES scintillation cocktail

Procedure
Sample Collection

1. Immediately before sampling, break the ^{14}C ampoule and decant into a clean 20 ml scintillation vial. Carefully wrap vial with kimwipes and place into a 1000 ml HDPE bottle for transport (store upright).

2. In a darkened environment, decant water from the Niskin bottle into 2-light and 1-dark 125 ml Teflon screw-cap bottles (rinse 3x with sample water before filling with sample). Replace bottles into their carrier until all PPR depths are collected. **Note**: Insert the hose to the bottom of the bottle and fill from the bottom up, overflowing the bottle with ~50 ml of sample.

3. Once all of the PPR bottles are filled, inoculate each sample with ^{14}C as follows. Arrange the radioisotope safety tray such that two people may work together during this procedure. One person will add the isotope while the second person thoroughly mixes each sample and replaces it into the carrier. Firstly, decant enough sample so that when the isotope is added there is about 1 cc of headspace in the bottle. Secondly, withdraw an appropriate volume of isotope (Table 9), place the pipet tip below the surface and dispense. Securely replace cap and gently invert sample three times. The second person will check the tightness of the cap and gently invert sample another ten times before replacing into carrier.

Table 9. Volume of ^{14}C bicarbonate working solution (100-120 μCi ml^{-1}) added to each sample bottle (15 ml ampules are used for Lake Bonney; 10 ml ampules are used for Lake Haore and Fryxell; 5 ml ampule for Lake Miers).

West Bonney		**East Bonney**		**Hoare**		**Fryxell**	
Depth (m)	^{14}C (μl)	Depth (m)	^{14}C (μl)	Depth (m)	^{14}C (μl)	Depth (m)	^{14}C (μl)
4.5	220	4.5	220	4.5	200	4.5	230
5	220	5	220	5	200	5	230
6	220	6	220	6	200	6	230
8	220	8	220	8	200	7	230
10	220	10	220	10	200	8	470
12	650	12	400	12	310	9	470
13	650	13	700	14	310	10	500
14	650	15	700	16	430	11	500
15	650	18	700	18	430	12	500
17	650	20	700	20	430		
20	650	22	700	22	430		

Total ^{14}C	**15000**	**15000**	**10020**	**10080**

Miers

Depth (m)	^{14}C (µl)
5	100
7	100
9	100
11	100
13	100
15	200
16	200
17	200
18	200
19	200
Total ^{14}C	**4500**

Note: Use the same calibrated 1000 µl Gilson for all isotope additions.

4. During sample deployment use an opaque tarpaulin to cover the incubation hole and sample carrier, thus preventing any direct sunlight from shining on the bottles. Carefully attach each bottle to the calibrated incubation line (the dark bottle on the lower clip, and the two light bottles on the upper clip at each depth, with a cable tie securing the loop on each bottle to the cable in case a hook should break) and slowly lower into the lake**.** Position the PPR incubation line in the center of the hole to prevent the incubation line from freezing into the sides of the hole. Secure the incubation line to a stout cane pole (or chipper bar) so that the piezometric mark on the line is even with the piezometric water level in the hole. Attach the incubation line to a secure point on the surface of the ice (i.e., hole melter, ice screw). Keep the hole covered with a tarpaulin during the incubation period.

Note: Always attach/detach the PPR bottles to the incubation line while the bottle is in the carrier, and begin with the deepest depths first, then lower the line into the hole as the shallower depths are attached.

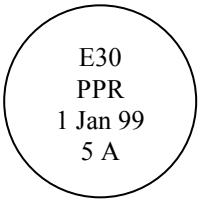
5. PPR Incubations should start by 7:30 am and continue for 24 h. **Note**: It takes about 2.5 h to collect samples before incubations begin. **Hence, collection should begin at 5:00 am.**

6. After the 24 h incubation, remove the bottles from the lake and place into the transport carrier. Two people are required to remove the bottles, one person will slowly remove the bottles from the lake, being careful not to hit the bottom or sides of the ice hole. Only retrieve one depth at a time; place the bottles over the transport carrier before detaching each one from the line. The other person will attend to the incubation line and assist.

Below steps to be conducted in Dry Valleys Lab

Sample Analysis

1. Label 3 scintillation vials per depth (A, B, D). Label the cap on the vial as follows:



2. Place 25 mm Whatman GF/F filters on the filter base and replace tower (use only rad labeled filtering equipment).

3. Pour sample into filter tower and filter under low pressure (< 7 inches Hg). Make sure tower is securely tightened down, it helps to add a small amount of the liquid at first in case there are any leaks so you don't lose the entire sample, then add any remaining sample. Remove filter and place (organic side up) in the bottom of the scintillation vial and cap. **Note:** Take precautions not to misplace or exchange samples among depths.

4. Once all of the samples are filtered, remove the caps and place scintillation vials on a heat block (60° C). To prevent possible misidentification of samples, position the caps so they directly correspond to the order of scintillation vials, and use a Sharpie marker to label each scintillation vial on the shoulder of the vial.

5. Using an Eppendorf repeater pipet, add 0.5 ml of 3N HCl to each scintillation vial (Inside fume hood).

6. Dry filters slowly (8 h at 60 °C) on heating block in fume hood. Once the filters are dry, carefully remove from the heat block and replace cap. (Filters can be removed from heat before 8 hours if they are dry).

7. Package scintillation vials and transport to MCM.

Below steps to be completed in MCM Crary Lab

8. Using a repipettor, add 10 ml of Cytoscint to each sample and count using the calibrated Beckman LS6000 Scintillation counter (Priscu PPR channel). The counter should be calibrated using ^{14}C-toluene Quench standards each season.

9. Primary production ($\mu g\ C\ l^{-1}\ d^{-1}$) is calculated using the following equation:

$$\mu g\, C\, l^{-1} d^{-1} = \left(\frac{\left(DPM_L - DPM_D\right) \cdot a \cdot b \cdot c}{A_{14_C} \cdot \left(\frac{Vol\ ^{14}C(\mu l)}{c} \right) \cdot \left(\frac{2.2 \times 10^6\, dpm}{1 \mu Ci} \right) \cdot t} \right) \cdot \left(\frac{24h}{d} \right)$$

where DPM_L is the average dpm of the light bottles, DPM_D is the dpm of the dark bottle, a is the concentration of dissolved inorganic carbon at the respective depth, b is the isotopic discrimination factor of ^{14}C radiolabeled carbon (1.06), c is a constant to convert units (1000), A_{14C} is the specific activity of the ^{14}C, and t is the incubation period (h).

Bacterial Enumeration and Biomass

General Discussion

SYBR® Gold was recently found to have a high quantum yield of fluorescence and less fade than Acridine orange (Lisle and Priscu, 2004). The following method has been used by Dr. Priscu in these lakes since 1999(?), and its continued use ensures consistency. It is important to take special care handling samples from the time of water collection to filtering in order to minimize contamination.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

Glass scintillation vials (20 ml with HDPE cone caps)
10ml Gilson Pipetman
Autoclaved 10 ml pipet tips (autoclave 20 minutes)
Acrodisc 0.2 µm filters and syringe

MCM Crary Lab/MSU

0.2 µm 25 mm black polycarbonate filters
0.45 µm 25 mm membrane filters
Acrodisc 0.2 µm filters and syringe
P1000 & P200 Gilson Pipetman
Autoclaved 1 ml pipet tips
1 ml syringe and sterile 23G1 needle
Filter manifold and glass filter base/tower
3 ~1000 ml containers for soaking filter towers (Alconox, 10% HCL, DIW)
Squirt bottle with 95% ETOH
Glass cover slips, 25x25 mm
Glass slides
Immersion oil (ultra low fluorescence grade)
Microscope with mercury lamp (100 W lamp is best), UV filter set, 100× objective or greater (SYBR Gold excitation maxima = ~495 and 300 nm; emission maximum – ~537 nm). See Appendix 6.8 for instructions on the Nikon Labophot scope with HBO 100W/L2 mercury lamp used by the Priscu Lab.
Microscope camera system (if available)
Permanent marker
Water (0.2 µm filtered reverse osmosis or distilled)

Reagents

Dry Valleys Lab

Formalin: (0.2 µm filtered) Buffer by saturating formalin with sodium borate.

MCM Crary Lab/MSU

SYBR® Gold Nucleic Acid Gel Stain (10,000X concentrate in DMSO)
TBE buffer

Antifade solution (0.1% p-phenylenediamine in a 1:1 solution of PBS/glycerol)
PBS buffer (currently we are using packets that are dissolved in DIW)

Procedure
Sample Collection
1. Fill a 1000 ml HDPE amber bottle (rinse 3x with sample water before filling with sample) with 1000 ml of sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport.

Below steps to be conducted in Dry Valleys Lab
2. Bacteria samples are taken from the 1000 ml Amber HDPE bottles. Gently invert the amber bottle to thoroughly mix, remove sample cap and pipet 18 ml of sample into a clean 20 ml glass scintillation vial, replacing cap immediately. Preserve all bacteria samples by adding 0.9 ml (~27 drops from Acrodisc syringe filter) of buffered formalin (0.2 µm filtered) to each sample. Store at 4ºC until ready to prepare samples for counting. Do not freeze during transport. ***Note:*** Storage for extended periods reduces cell counts. We count cells within 3 months of sample collection.

Below Steps to be done in MCM Crary Lab and/or MSU
1. Prepare a 10X solution of TBE buffer by dissolving in 500 ml *DI* water: 54g Tris Base, 27.5g boric acid, 20 ml 0.5M EDTA (pH 8.0). Dilute to 1X and filter through a 0.2 µm acrodisc into a 50 ml sterile, non-pyrogenic, polystyrene tube. Store at room temperature.

2. Prepare a 25X SYBR Gold solution: Thaw SYBR® Gold Nucleic Acid Gel Stain (10,000X concentrate in DMSO) and briefly centrifuge suspension to separate the dye from the DMSO (DMSO will be deposited at the bottom of the tube). Be sure the dye solution is fully thawed before removing an aliquot. Add 25 µl of dye to 9.975 ml of filtered 1X TBE buffer. Vortex. Filter solution through a 0.2 µm acrodisc and into a 15 ml sterile, non-pyrogenic, polypropylene tube. Seal cap with tape so that it is clear whether tubes have been previously used. Wrap tube in aluminum foil (SYBR Gold is light sensitive) and refrigerate at 4ºC. 25X SYBR Gold solution can be used for up to 5-7 days. (SYBR Gold solution must be poured through activated charcoal prior to disposal). See www.molecularprobes.com for more information on SYBR® Gold Nucleic Acid Gel Stain.
Caution: No data are available addressing the mutagenicity or toxicity of SYBR® Gold nucleic acid gel stain. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

3. Immediately before slides are to be made, prepare a solution of Antifade (0.1% p-phenylenediamine in a 1:1 solution of PBS/glycerin) by weighing 0.1g of p-phenylenediamine into a 15 ml sterile, non-pyrogenic, polystyrene tube. Add 1 ml 0.2 µm filtered DI water and vortex until dissolved. Add 4.5 ml glycerol and 4.5 mls phosphate buffered saline (PBS) and vortex. Filter solution through a 0.2 µm acrodisc into a 15 ml

sterile, non-pyrogenic, polystyrene tube. Wrap tube in aluminum foil (Antifade solution is light sensitive) and refrigerate at 4°C). Antifade solution is only good for ~12 hours (solution will turn a brown color after this time), and must be prepared fresh each day slides are to be made.

4. Place a 0.45 μm 25 mm diameter membrane filter on a glass fritted filter apparatus base and cover with a 0.2 μm 25 mm diameter black polycarbonate filter (shiny side up). Replace filter tower (scrubbed with Alconox, soaked in 10% HCL, rinsed in DIW, rinsed with 95% ETOH).

5. Invert 20 ml glass scintillation vial containing sample, and add appropriate volume of sample to filter tower (2 ml for Lakes Hoare and Fryxell, 3 ml for Lake Bonney. These volumes can be changed depending on the amount of cells present in the sample; generally the lower depths of Bonney need 5-6 mls). Add 500 μl of 25X Sybr Gold nucleic acid stain (adjust if filtering more sample). Allow sample to incubate in the dark (foil over tower) for 15 minutes before filtering.

6. Filter under low vacuum (0.3 atm; 5 inches Hg). Just as a thin layer of sample remains, rinse filter tower with 1 ml of filtered *DI* water and continue to filter. Turn off vacuum as soon as the last of the water has filtered.

7. Place filter on a labeled glass microscope slide containing 1 drop of Antifade solution. Place two drops of Antifade solution, using a 1 ml syringe, on top of filter. Place a cover slip on top of filter.

8. Use a clean and blunt object to gently push down on the cover slip in order to form a thin even film of Antifade solution between the filter and the cover slip. There should be no bubbles, and the filter should not have any wrinkles. Freeze upright in a slide holder until ready to count.

9. Prepare a sample blank by following the above procedure using filtered DI water (without sample). This should be done prior to filtering samples, and checked on the microscope to assure there is no contamination in any of the solutions.

10. Place a small drop of immersion oil on each cover slip. Count bacteria at a final magnification of 1000× in at least 10 different fields and until at least 300 cells are counted, yielding a < 15% counting error. If 300 cells have not been counted after counting 30 fields, the number of cells counted thus far is sufficient. At least one digital image of each depth should be taken for archiving purposes (a picture of the stage micrometer will need to be taken for calibration of the camera).

Calculation of Cell Numbers

1. Calculate cell numbers (cells ml^{-1}) where *S* is the average number of cells per field in the sample, *B* is the average number of cells per field in the blank, *vol* is ml of sample filtered,

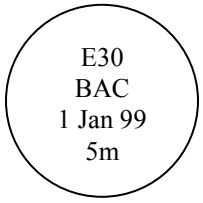
filter area is the area (μm^2) of the filter containing bacterial cells (determined by measurement of the filtration "spot" (dye area) on the filter), *field area* is the area for each field counted (μm^2).

$$\text{cells ml}^{-1} = \left(\frac{S-B}{vol}\right)\left(\frac{filter\ area}{field\ area}\right)$$

Notes on Bacterial Protocol

1. Always wash your hands and wear latex/vinyl gloves when labeling scintillation vials, handling pipet tips or preparing bacteria samples.

2. Label scintillation vials before preservation, preferably the night before sampling so that samples may be prepared immediately following lake water collection. Use only unopened scintillation vial packs. Once the plastic is removed from a flat of vials, immediately cap these vials or don't plan on using them on any bacteria work. The best way to cap the vials is to cut the plastic on the far edge of the flat and fold it over. Place caps on vials one row at a time, folding the plastic back as you progress through the rows. Take care to not touch the inside of the caps with your fingers. Label vials accordingly:

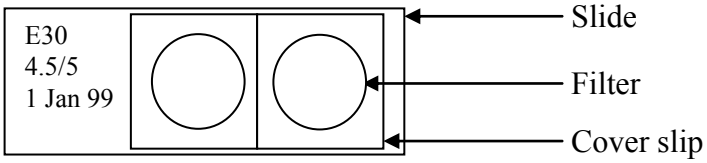


3. Use a P10ml Gilson Pipetman and 10 ml pipet tips. To autoclave these tips, place 20 tips with pointy side down into an autoclavable bag (8 in × 11in), tape closed with autoclave tape, and autoclave for 20 minutes.

4. A Hoefer 10 place manifold with glass towers works well.

5. Clean and scrub filter apparatus with a brush between filtering samples. Rinse well with *DI* water, followed by 0.2 μm filtered water.

6. Two filters may be placed on one microscope slide



7. Counting bacteria may seem ambiguous at first, but as you become familiar with the different types of bacteria, consistency can easily be achieved.

   Some cells look like bacteria, but have chlorophyll and therefore will autofluoresce. These cells are red, not orange or green. Sometimes this is hard to distinguish. Look at any questionable cells under UV light. If the cells are visible under UV, then they are autofluorescent and should not be counted.

   Generally, rods look like this ⬬, and cocci like this ●, but you will see cells shaped like this ∽, especially in the deeper waters of Bonney and Fryxell. If cells are long (>5 μm) and string like, they are filaments. If they do not autofluoresce then they should be counted. Move randomly around the filter counting cells in each field (or within the Whipple disk if cell number is high enough), and record the numbers of filaments, rods, and cocci. Count at least ten fields. If 300 cells have not been counted after 10 fields, continue counting until 300 cells have been counted. If 300 cells have not been counted after counting 30 fields, the number of cells counted thus far is sufficient. Individual records should be kept of rods, coccoid and filamentous cells so that cell numbers and biovolume can be computed for each type of bacteria.

8. Shipping considerations:
   Check each scintillation vial and make sure the cap is tightened well. Cover each flat with a piece of cardboard and tape it down. Place upright in a freezesafe and secure with bubble wrap to prevent movement during shipping. Bacteria samples must **NOT FREEZE**, handle samples appropriately. Although these samples contain formalin, they are not hazardous cargo. The final concentration of Formaldehyde is <2%.

Biovolume Measurements

Biovolume measurements are no longer performed (since 0203 season). The Biovolume method previously used is as follows:
Measure length and width of cells in one randomly chosen field to determine biovolume. Alternatively, pictures can be taken using a microscope camera system, such as the Optronics Microfire digital camera system used at MSU, and cell sizes measured later using the Sigma Scan program (a picture of the stage micrometer will need to be taken for calibration of the camera).

Biovolume is calculated using the following formulas for rods (ellipsoid) and coccoid (sphere) cells, respectively:

$$\text{Ellipsoid} = \frac{\pi AB^{2}}{6} \qquad \text{Sphere} = \frac{\pi A^{3}}{6}$$

where A is the length and B is the width of the ellipsoid cell, and A is the diameter of the spheroid cell.

Bacterial Degradation Equation

Based on a time series experiment with SYBR Gold performed from November 2004 – January 2006, it was determined that bacterial counts need to be corrected for cell degradation from the time of collection/preservation to counting (Figure 4).

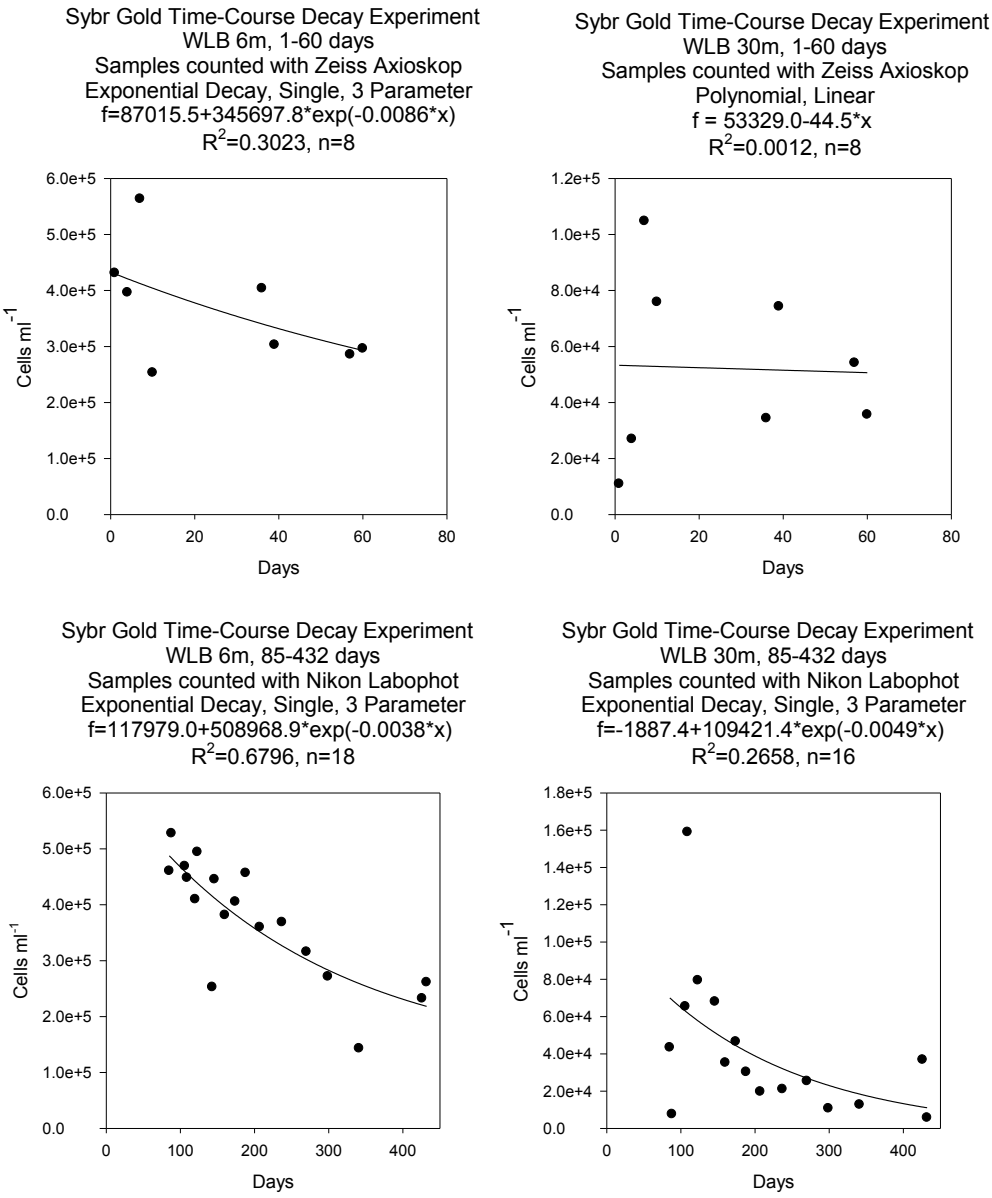


Figure 4. Cells per ml plotted over time for West Lake Bonney samples counted for the time-course decay experiment for SYBR Gold dye.

The degradation equation previously used for Acridine Orange is as follows:
Correct bacterial counts for cell degradation from the time of collection/preservation to counting using the following equation:

$$N_t = N_0 e^{-0.0037t}$$

where N_t is the number of cells counted at time t, time t is the time elapsed in days, and N_0 is the number of cells in the sample at time zero (Takacs and Priscu, 1998).

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Bacterial Production Determined by ^{3}H Thymidine and ^{3}H Leucine Incorporation

General Discussion

Despite the controversy over the shortcomings of the thymidine incorporation method, its widespread use in various systems, the relative simplicity of the method, and its repeatability make this a useful technique to determine bacterial production. During the 0607 season, we did a comparison of the "filtration method" used in previous seasons (see Appendix; Takacs, C.T. and J.C. Priscu. 1998. Bacterioplankton dynamics in the McMurdo Dry Valley lakes: Production and biomass loss over four seasons. Microbial Ecology 36:239-250), with the "centrifugation method" used by the Palmer (PAL) LTER (Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Marine Biology 66:109-120). Results showed no significant difference between the two methods (ELB 0-20 m: mean and standard deviation of nM TDR/d determined via filtration method, 0.0113 and 0.0091, respectively; mean and standard deviation of nM TDR/d determined via centrifugation method, 0.0124 and 0.0122, respectively; paired t-test T value, -0.7332; P value, 0.4911). We will therefore continue this measurement using the centrifugation method during future seasons. We will also complement our thymidine data with 3H-leucine incorporation data, as is done in the PAL LTER.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

2 ml microcentrifuge tubes (VWR # SCT-200) (autoclave 20 minutes)
Tube racks
Permanent marker
Eppendorf repeater pipet and sterile 10 ml tips (these cannot be autoclaved – buy sterile tips, which come in bags of 10)
p10 Gilson Pipetman (rad use only)
Sterile 10 µl pipet tips
p100 Gilson Pipetman (rad use only)
Autoclaved 200 µl pipet tips (autoclave 20 minutes)
P1000 Gilson Pipetman (rad use only)
Autoclaved 1000 µl pipet tips (autoclave 20 minutes)

MCM Crary Lab

Permanent marker
Centrifuge (14,000 rpm; ~15,000 x g)
Eppendorf repeater pipet and 10 ml tips
10 ml Repipettor for cocktail in fume hood
Liquid scintillation counter

Reagents

Dry Valleys Lab

^{3}H-Thymidine (20 Ci mmol^{-1}): precursor of DNA, uptake indicates rate of DNA synthesis
^{3}H-Leucine (40-60 Ci mmol^{-1}): amino acid, uptake indicates rate of protein synthesis
Trichloroacetic acid (100% TCA): Bring 20g of TCA up to 20 ml with DIW.
MCM Crary Lab
Trichloroacetic acid solution (5% TCA): Dissolve 50g of TCA in *DI* water, and bring volume up to 1000 ml. Store at ~1ºC
Ethanol solution (80%)
Cytoscint ES scintillation cocktail

Procedure
Sample Collection
1. Fill a 1000 ml HDPE amber bottle (rinse 3x with sample water before filling with sample) with 1000 ml of sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport.

Below steps to be conducted in Dry Valleys Lab
1. Label five- 2 ml micro centrifuge tubes per depth, preferably the night before sample collection. Two of the five vials are kills and should be labeled K1 and K2. Three of the vials are live treatments and should be labeled T1-T3. Note that not all depths (m) are incubated for all lakes (Table 10).

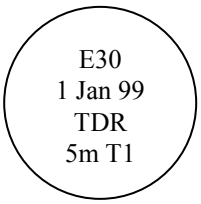

Table 10. Depths incubated for bacterial production experiments.

Fryxell (m)	Hoare (m)	Miers (m)	ELB (m)	WLB (m)
4.5	4.5	5	4.5	4.5
5	5	7	5	5
6	8	9	10	10
8	12	11	13	13
9	14	13	15	14
10	16	15	18	15
11	20	16	20	17
15	25	17	25	25
18	30	18	30	30
		19		

2. The thymidine stock solution comes in ethanol which eliminates bacterial growth and volatile products of self-radiolysis. Results from experiments done during the 0405 season indicate that it is not necessary to evaporate the ethanol from solution as was the procedure in past years. Radiolabeled thymidine is added to each vial to achieve a final concentration of 20 nM. If the specific activity of the ^{3}H Thymidine stock is 20 Ci mmol^{-1}, then .6 µl of stock solution must be added to each vial (See calculation of ^{3}H Thymidine addition at the end of this section). In order to achieve an easier pipetting volume, the thymidine stock solution is first diluted10-fold in DIW (0.1 ml (100 µl) thymidine :0.9 ml (900 µl) 0.2 µm filtered DIW). 6 µl of the diluted solution is then added to each vial.

3. Radiolabelled leucine is added to each vial to achieve a final concentration of 20 nM. If the specific activity of the ^{3}H Leucine stock is 84 Ci mmol^{-1}, then 2.52 µl of stock solution must be added to each vial (See calculation of ^{3}H Leucine addition at the end of this section). In order to achieve an easier pipetting volume, the leucine stock solution is first diluted10-fold in DIW (0.1 ml thymidine :0.9 ml 0.2 µm filtered DIW). 25.2 µl of the diluted solution is then added to each vial.

4. Add radioisotope-labeled thymidine or leucine to live tubes (6 µl diluted thymidine; 25.2 µl diluted leucine) using the appropriate Pipetman. Dispense isotope drop of isotope well within vial, touching inside wall with tip to ensure complete dispensing of isotope from tip. Don't add isotope to blank (kill) tubes yet (see below). After inoculation, cap tubes loosely.

5. Bacterial production samples are taken from the 1000 ml amber HDPE bottles. Gently invert the amber bottle to thoroughly mix, withdraw 9-10 ml of sample using the Eppendorf repeater pipet with a 10 ml tip, and dispense 1.5 ml into all tubes (live and kill), replacing cap immediately. Pipet water carefully to avoid splashing sample and isotope out of tubes. Use 1 tip per depth (9 total for each lake), which will leave you 1 tip from the sterile bag for TCA addition. **Record time of addition of water to isotopes.** (Note: you will incubate the samples for 20hrs so plan your inoculation accordingly. For example it is best to kill the samples around 10am the next day so you'd want to start the incubation around 2pm). Cap live tubes firmly, and invert several times.

6. Perform activity checks of the thymidine and leucine by pipeting 3 µl from each of five live thymidine and leucine treatments (any depths) into 2 ml tubes, using the p10 Pipetman. Cover rack of samples with aluminum foil (to keep dark) and place live samples (and activity checks) in incubator at 4 ºC.

7. Add 100 µl of cold 100% TCA to each control (kill) tube using the Eppendorf repeater pipet and 10 ml tip. Cap, invert several times, and place on ice for 15 minutes. Carefully add isotope to dry area on inside of tube near top using the appropriate Pipetman. Do not touch pipet tip to the sample containing the TCA. Cap the vials firmly, invert several times, and place in incubator with live samples (covered in aluminum foil).

8. Incubate the samples in the dark at 1-4ºC for 20 h. Monitor temperature using Stowaway temperature loggers, if it varies, compute a time weighted average temperature for the incubation period.

9. Terminate incubation with the addition of 100 µl of cold 100% TCA to each live sample using the Eppendorf repeater pipet. Invert tubes several times and store on ice for 15-30 minutes. **Record time of TCA addition.** Store vials in dark at 4ºC until processed.

Below steps to be conducted in MCM Crary Lab

10. Centrifuge tubes at 14,000 (~15,000 x g) for 15 minutes. Load tubes with marker tab facing out, and do so for all subsequent centrifugations.

11. Gently pour out the supernatant into a wide-mouth waste jar and gently tap lip of each tube on a paper towel to get the last drops of hot liquid off. Pour with the marker tab on tube at the top.

12. Add 1 mL of cold 5% TCA (use Eppendorf repeat pipettor) to each tube. Re-spin for 5 minutes, loading tubes with marker tab facing out.

13. Gently pour out the TCA and gently tap the lip of each tube on a paper towel to get the last adhering drop out.

14. Add 1 mL of cold 80% ethanol (use Eppendorf repeat pipettor) to each tube. Re-spin for 5 minutes, loading tubes with marker tab facing out.

15. Gently pour out the ethanol and gently tap the lip of each tube on a paper towel to get the last drops out.

16. Place open tubes in fume hood until dry (overnight). **No ethanol can be left in the tubes.** It is a quencher!

17. Once the samples are dry, add 1 ml of Cytoscint ES or scintillation cocktail (use Eppendorf repeat pipettor), vortex well, and count samples in a liquid scintillation counter on a calibrated ^{3}H channel. Add cocktail to activity check samples and count as well.

18. Thymidine and Leucine uptake rate is determined by:

$$\text{nM (TdR or Leu) day}^{-1} = \left(\frac{(DPM\ treatment - DPM\ Kill)(nM\ thy, leu)}{(\mu Ci)\left(\frac{2.2\text{x}10^{6}\ dpm}{1\mu Ci}\right)(t\ hr)} \right) \cdot \left(\frac{24hr}{d} \right)$$

where *DPM treatment* is the average DPM of T1-T3, *DPM kill* is the average treatment of the kills, *nM thy*,*leu* is the final concentration of thymidine or leucine in the incubation vial (20 nM), μCi is the activity added (4 μCi), 2.2×10^6 is the number of dpm μCi^{-1}, and *t* is the incubation time (h).

16. Thymidine and Leucine uptake rate is corrected for the incubation temperature as follows:

$$\text{nM (TdR}_t \text{ or Leu}_t\text{) day}^{-1} = \text{nM (TdR or Leu) day}^{-1} \cdot e^{\left(\frac{Ea \cdot \left[\left(\frac{1}{^\circ C_I + 273^\circ K}\right) - \left(\frac{1}{^\circ C_A + 273^\circ K}\right)\right]}{R}\right)}$$

where Ea is the energy of activation of 12,600 kcal mol^{-1}, $°C_I$ is the incubation temperature (°C), $°C_A$ is the ambient lake water temperature at specific depth (°C), and R is a gas constant (1.987 cal mol^{-1} $°K^{-1}$), (Priscu, unpublished data).

17. Thymidine uptake rate is converted to bacterial production by empirically determining a carbon conversion factor for the assemblage being studied (Kirchman and Ducklow, 1993). There are a number of carbon conversion factors reported in the literature to convert thymidine and leucine uptake rate to bacterial production. The MCM LTER uses the following conversion factors (Takacs and Priscu, 1998; Kirchman, 1988):

Thymidine:
2.0×10^{18} cells mol thymidine^{-1}
11 fg Carbon cell^{-1}.

Leucine:
1.420×10^{17} cells mol leucine^{-1}
11 fg Carbon cell^{-1}.

Calculation of ^{3}H Thymidine addition

In order to compute the volume of 3H-thymidine to add to each experimental vial to yield a final concentration of 20 nM, one must first compute the concentration of thymidine in the radioactive stock received from the vendor. Assuming the ^{3}H stock solution has a specific activity of 20 Ci mmol^{-1} (specific activity will be noted on product), and an initial concentration of 1 mCi ml^{-1} the following calculations are used to compute the concentration of thymidine:

$$\frac{\text{mmol}}{20\,\text{Ci}}\left(\frac{\text{Ci}}{1000\,\text{mCi}}\right)=\frac{\text{mmol}}{20{,}000\,\text{mCi}}$$

$$\frac{\text{mCi}}{\text{ml}}\left(\frac{\text{mmol}}{20{,}000\,\text{mCi}}\right)=\frac{\text{mmol}}{20{,}000\,\text{ml}}=\frac{\text{mmol}}{20\,\text{L}}$$

$$\frac{\text{mmol}}{20\,\text{L}}\left(\frac{10^6\,\text{nmol}}{\text{mmol}}\right)=\frac{50{,}000\,\text{nmol}}{\text{L}}$$

This concentration of thymidine can then be used to compute the volume of radiolabeled stock to yield a final concentration of 20 nM in the 1.5 ml sample:

$$(C_I)(V_I)=(C_F)(V_F)$$

where C_I is the initial concentration of the stock solution, V_I is the volume of stock solution added to sample, C_F is the desired final concentration of stock solution in the sample, and V_F is the total volume of the sample.

$$\frac{50{,}000\,\text{nmol}}{\text{L}}(\text{X mls})=\frac{20\,\text{nmol}}{\text{L}}\quad(1.5\,\text{ml})$$

$$\text{X mls}=0.0006$$

$$\therefore\ 0.6\,\mu\text{l}\ (0.6\,\mu\text{Ci})\ \text{of stock solution should be added to each vial}$$

Because 0.6 µl of stock solution is not easily pipetted with great accuracy, we dilute the stock solution 10-fold and pipet 6 µl of dilute stock solution (0.1 ml thymidine : 0.9 ml 0.2 µm filtered DIW). Theoretically, 6 µl of the reconstituted solution will contain .6 µCi of ^{3}H thymidine.

Calculation of ^{3}H Leucine addition

In order to compute the volume of 3H-leucine to add to each experimental vial to yield a final concentration of 20 nM, one must first compute the concentration of thymidine in the radioactive stock received from the vendor. Assuming the ^{3}H stock solution has a specific activity of 84 Ci mmol^{-1} (specific activity will be noted on product), and an initial concentration of 1 mCi ml^{-1} the following calculations are used to compute the concentration of leucine:

$$\frac{\text{mmol}}{84\,\text{Ci}}\left(\frac{\text{Ci}}{1000\,\text{mCi}}\right)=\frac{\text{mmol}}{84{,}000\,\text{mCi}}$$

$$\frac{\text{mCi}}{\text{ml}}\left(\frac{\text{mmol}}{84{,}000\,\text{mCi}}\right)=\frac{\text{mmol}}{84{,}000\,\text{ml}}=\frac{\text{mmol}}{84\,\text{L}}$$

$$\frac{\text{mmol}}{84\,\text{L}}\left(\frac{10^6\,\text{nmol}}{\text{mmol}}\right)=\frac{11905\,\text{nmol}}{\text{L}}$$

This concentration of leucine can then be used to compute the volume of radiolabeled stock to yield a final concentration of 20 nM in the 1.5 ml sample:

$$(C_I)(V_I) = (C_F)(V_F)$$

where C_I is the initial concentration of the stock solution, V_I is the volume of stock solution added to sample, C_F is the desired final concentration of stock solution in the sample, and V_F is the total volume of the sample.

$$\frac{11905\,\text{nmol}}{\text{L}}(\text{X mls})=\frac{20\,\text{nmol}}{\text{L}}\ (1.5\,\text{ml})$$

$$\text{X mls} = 0.00252$$

$$\therefore\ 2.52\,\mu\text{l}\ (2.52\,\mu\text{Ci})\ \text{of stock solution should be added to each vial}$$

Because 2.52 µl of stock solution is not easily pipetted with great accuracy, we dilute the stock solution 10-fold and pipet 25.2 µl of dilute stock solution (0.1 ml leucine : 0.9 ml 0.2 µm filtered DIW). Theoretically, 25.2 µl of the reconstituted solution will contain 2.52 µCi of ^{3}H leucine.

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Major ions by ion chromatography

General Discussion

The dry valley lakes span about 4 orders of magnitude in salt concentrations. All of the lake surface waters are fresh, the deep water of Lake Fryxell is brackish, and the deep waters at Lake Bonney are hypersaline. The entire water column of Lake Hoare is fresh. Dedicated filtration equipment is used at each lake to try to minimize contamination given the wide range of salt concentrations between the lakes. Most of the lake samples need to be diluted before analysis due to their high salt content

Anions and cation are analyzed in separate runs on the IC.
The major anions are F^-, Cl^-, Br^-, NO_3^-, and SO_4^{2-}.
The cations are Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}.

Detailed information on operating the Dionex IC can be found in the Instrument manual and will not be repeated here. This section describes the methods used for sample collection and preservation. Information specific to the analysis of the LTER limno samples is also included.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

47 mm magnetic filter towers
47 mm, 0.4µm Nuclepore filters
125 ml WM HDPE bottle (Acid washed (1% HCL) for cations, DI washed for anions – SEE PROCEDURE BELOW)
Bell jars and vacuum pump
DI water squirt bottle
Filter forceps
Ziplock bags

MCM Crary Lab

Plastic autosampler vials and caps
DI water
Squirt bottle for *DI*
White (cations) and blue (anions) label tape
P1000 Gilson Pipetman and tips (2)
P5000 Gilson Pipetman and tips
Disposable micro beakers, 10ml

Reagents

MCM Crary Lab

Individual ion standards for ions of interest.
Check standards for anions/cations

Methanesulfonic acid
$NaHCO_3$ and Na_2CO_3 eluent concentrate
Ultrex or Optima nitric acid

Procedure
Before sampling prepare the 125ml Nalgene bottles in Crary labs and bag up for the individual lakes and limno runs. Always include an extra bottle.
Bottles for anion analyses are washed with DI water by rinsing 3 times, then receive blue label tape.
Bottles for cation analyses are acid rinsed with 1% HCL for up to 1 hour (fill half-way with 1% HCL, cap, let sit right-side up for ~1 hour, flip upside down for ~1 hour) and DIW rinsed 5 times, and then receive white labeling tape. 1% HCL can be re-used for other acid rinsing since the bottles are new.

Sample Collection
1. Fill a 1000 ml HDPE amber bottle (rinse 3x with sample water before filling with sample) with 1000 ml of sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport.

Below steps to be conducted in Dry Valleys Lab
2. Gently invert the 1000 ml amber HDPE bottle. 2-100 ml aliquots will be filtered (one for anions, the other for cations).

3. Place a 0.4 µm 47 mm Nuclepore filter on the filter base of a 47 mm magnetic filter tower mounted on a vacuum bell jar, and replace tower. Be careful to place the filter correct side up (shiny side up) and only handle the filter at the edge with forceps. The filters can be torn easily. When in doubt, throw it out! Place either a DI washed 125 ml HDPE bottle for the anion filtrate, or an acid washed 125 ml HDPE bottle for the cation filtrate, directly under the filter base.

4. Pour sample from amber bottle directly into the *DI* rinsed filter tower; no need to measure sample, just use the markings on the filter towers as a guide. Filter each sample under low vacuum (<10 in Hg). First filter 100 ml of sample into an acid rinsed (white tape) 125 ml wide-mouth bottle for the cation sample. When finished, replace the acid washed bottle with a *DI* rinsed (blue tape) 125 ml wide-mouth bottle for the anion sample. Use the same filter tower for both the anion and cation aliquots. Use a new filter between the cation and anion samples (of the same depth) if filtering is slow; otherwise, you can use the same filter for both. Maintain quality control by carefully rinsing filtration apparatus with DI water between sample depths.

5. Prepare at least one filtration blank for anions and one for cations by filtering DI water through the filter tower apparatus.

6. Store samples in labeled Ziploc bags at 4ºC.

Below steps to be conducted in MCM Crary Lab

1. Preserve the cation samples by adding 0.5 % v/v (0.5 ml acid in 100 ml sample) of concentrated Ultrex or Optima HNO_3. Do not acidify the blank. The anion samples are not preserved.

2. Store the samples in a cool dark place. They can be frozen, but avoid successive heating and cooling.

Sample Analysis (this method was updated in October 2007 by Kathy Welch with information about the new instrument being used. See the Appendix for information about the previously used instrument).

A Dionex DX-120 ion chromatography dual-column system (Dionex, Sunnyvale, CA, USA) is used for the major ion analyses. The system includes a single piston isocratic pump module, high-pressure Rheodyne injection valve with a 25 µl sample loop, Dionex high-performance cell with heater (the DS4 Detection Stabilizer), and Dionex AS-40 automated sampler. The system is configured for anion and cation analyses with a switching valve to switch between columns. The eluent flow rate is set to 1.2 ml/min for both anion and cation analysis. Dionex Chromeleon software is used to automate the system, manage chromatograms and analyze data.

A Dionex IonPac CS12A analytical column (4x250mm) and a CG12A guard column (4x50mm) are used for cation analysis. The eluent is 0.13% methanesulfonic acid solution. A CSRS Ultra II Cation Self-Regenerating Suppressor was used. The background conductivity is approximately 200-250 nS.
For the anions a Dionex IonPac AS14 analytical column (4x250m) and an AG14 guard column (4x50mm) is used. The eluent is a 1.0mM $NaHCO_3$ and 3.5mM Na_2CO_3 solution. An ASRS Ultra II Anion Self-Regenerating Suppressor is used. The background conductivity is approximately 16 µS.

Standards

Cation and anion stock standards used for a typical batch of samples from the lakes are shown in Tables 11 and 12.

Table 11. Concentrations of cation standards used for calibration (mg/L).

	Li^+	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
Stock std	1	100	10	20	50	50
std6	0.5	50	5	10	25	25
std5	0.2	20	2	4	10	10
std4	0.1	10	1	2	5	5
std3	0.05	5	0.5	1	2.5	2.5
std2	0.02	2	0.2	0.4	1	1
std1	0.01	1	0.1	0.2	0.5	0.5

Table 12. Concentrations of anion standards used for calibration (mg/L)

	F^-	Cl^-	Br^-	NO_3^-	PO_4^-	SO_4^-
Stock std	10	100	2	2	2	100
std6	5	50	1	1	1	50
std5	2	20	0.4	0.4	0.4	20
std4	1	10	0.2	0.2	0.2	10
std3	0.5	5	0.1	0.1	0.1	5
std2	0.2	2	0.04	0.04	0.04	2
std1	0.1	1	0.02	0.02	0.02	1

Due to the high salt concentrations of many samples, dilute samples before analysis (Table 13). Lake water dilutions range from 1:2 for surface water of Lake Hoare to 1:5500 for deep water of Lake Bonney. Dilute samples by serial dilution using disposable plastic micro-beakers and adjustable pipettors. The stream samples can be run without dilution.

Table 13. Lake water sample dilutions for each lake.

West Bonney			**East Bonney**			**Hoare**			**Fryxell**		
Depth	Anion	Cation	Depth	Anion	Cation	Depth	Anion	Cation	Depth	Anion	Cation
4.5	10	10	4.5	10	10	4.5	10	10	4.5	10	10
5	10	10	5	10	10	5	10	10	5	10	10
6	10	10	6	10	10	6	10	10	6	10	10
8	20	10	8	20	20	8	10	10	7	10	10
10	100	100	10	100	100	10	10	10	8	55	55
12	200	100	12	200	100	12	10	10	9	100	100
13	200	100	13	200	100	14	10	10	10	100	100
14	1000	1000	15	550	100	16	10	10	11	100	100
15	2000	1000	18	1000	1000	18	10	10	12	100	100
17	2000	1000	20	5500	2000	20	10	10	15	100	100
20	2000	1000	22	5500	2000	22	10	10	18	100	100
22	2000	1000	25	5500	2000	25	10	10			
25	2000	1000	30	5500	2000	30	10	10			
30	2000	1000	35	5500	2000						
35	2000	1000	38	5500	2000						
39	2000	1000									

1. Every 10th sample should be duplicated to check the precision of the dilutions. The percent relative standard deviation (%*RSD*) of the duplicates should be less that 1%, even with dilutions of 1:5500.

$$\%RSD = \frac{SD}{\bar{X}} \cdot 100$$

From an analytical perspective, these lakes pose a few problems for analysis. Cl^- concentrations in the deep waters of the east lobe of Lake Bonney are ~180,000 mg l^{-1}. That is approximately 10 times the Cl- concentration of seawater. All of the sampling equipment and filtration equipment must be rinsed thoroughly to prevent carryover of salts from one sample to the next. In addition, these samples require extensive dilution before analysis.

Dedicated sampling equipment for each lake and for the streams is essential for maintaining the integrity of the samples. For example, the range of Cl^- concentration in the lakes under investigation varies by more than 3 orders of magnitude and carryover between samples becomes a potential problem. Carryover can also be a potential problem during analysis and, therefore analytical blanks are run to monitor this. In general, if care is taken during sample processing and analysis, very good analytical results can be obtained.

References

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Dissolved Inorganic Carbon by Infrared Gas Analyzer

General Discussion

This section describes the collection, preservation and analysis of dissolved inorganic carbon (DIC) based on sample acidification followed by infrared gas analysis and comparison to standard curves for determination DIC concentration. Infrared gas analysis is necessary because alkalinity based DIC is affected by high levels of H^+ accepting compounds other than HCO_3^-/CO_3^{-2}. Also, DIC is used in the calculation of primary production.

Materials

Sampling Hut

30 ml serum vials (Bottles from the previous year can be re-used: check for salt build-up before using. If they are clean, rinse with 1% HCL, then rinse 6X with DIW. It is best to clean bottles at the end of the season to avoid salt build-up for the next year. If bottles are brand new, they can be DIW rinsed 6X before use.)
Serum/Scintillation vial carrier
P200 Gilson Pipetman and tips
Crimping tool
Rubber stoppers and aluminum crimping caps

MCM Crary Lab

250 ml volumetric flask
500 and 1000 µl Gastight Syringe with Luer-lock tip
24 ga.-1 ½ in. needles
Qubit system *(previously a Lira IRGA with HP Integrator or Licor 6252 CO2 analyzer was used. These systems were replaced with the Q-bit during the 0708 season)*
Peak Simple *(we no longer use the HP Integrator so these methods have been deleted)*

Reagents

Sampling Hut

Chloroform

MCM Crary Lab

6 N H_2SO_4
Sodium Bicarbonate ($NaHCO_3$)
Anhydrone (Magnesium perchlorate)
Nitrogen gas

Procedure

Note: It is best to put chloroform in a scintillation vial and then place this inside a 125ml Nalgene for transport.

Note: Be sure to ventilate the polarhaven while using the Chloroform and to change your gloves if any Chloroform gets on them. Chloroform will go through both Latex and Nitrile gloves!

Sample Collection

1. Rinse each serum vial 3x with sample water before filling. Place the Niskin bottle tubing from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times) to the bottom of the serum vial and allow the water to overflow, displacing the initial sample volume 2x (about 5 seconds). Carefully remove the tubing keeping turbulence to a minimum, making sure the vial is completely filled.

2. Immediately preserve the sample with 0.15 ml chloroform, cap and crimp the bottle. Place sample into the Serum/Scintillation vial carrier for transport.

3. Store the samples in the dark at 4°C until analysis.

Below steps to be conducted in MCM Crary Lab

4. Assemble the CO_2 analyzer (Figure 5). Place new Ascarite (CO_2 filter) and Anhydrone (magnesium perchlorate – dessicant (use 70% Anhydrone + 30% teflon boiling stones to help prevent the Anhydrone from clumping)) into the scrubber cylinders (cylinders should be mounted vertically, with the gas entering from the base). Always replace the Anhydrone and 6 N H_2SO_4 before proceeding to another batch of samples. Recalibration via a new standard curve is necessary following replacement.

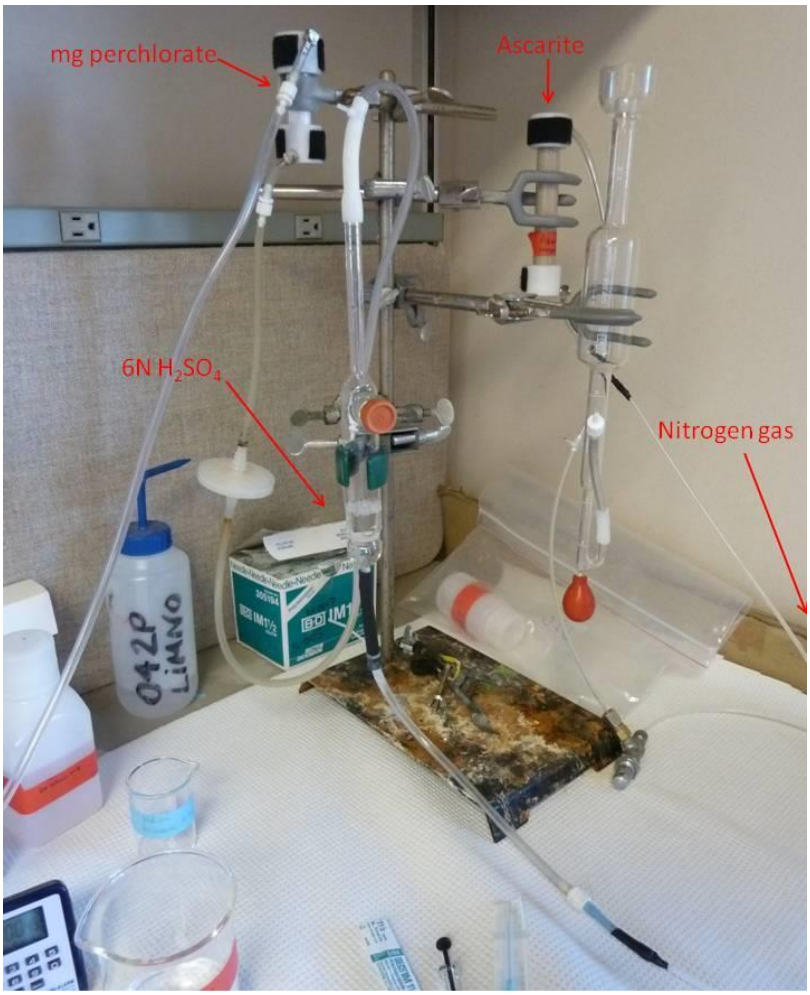


Figure 5. Set up of the DIC analysis system.

5. Turn on the Qubit (Figure 6), set to the 2000 ppm range, and allow it to warm up for 90 minutes before analyzing samples. The Qubit can be turned on and left on for the period of time (days) that samples are being run.

Figure 6. Qubit

6. Prepare a 60 mg l^{-1} DIC standard by bringing 0.105 g $NaHCO_3$ to 250 ml with *DI* water in a volumetric flask. $NaHCO_3$ should first be heated to drive off any moisture by placing in a glass beaker, heating in a drying oven at 110° C for at least 1 hour, and cooling in a dessicator. Following preparation of standard solution, decant into a bottom arm flask and stopper. This standard can be used for up to 2 days, as long as the slope of the standard curve stays consistent.

$$\frac{0.1050\text{g } NaHCO_3}{250\text{ ml DIW}} \bullet \frac{12.01\text{ g/mol C}}{84.005\text{ g/mol } NaHCO_3} \bullet \frac{1000\text{ ml}}{1\text{ L}} \bullet \frac{1000\text{ mg}}{1\text{ g}} = 60.05\text{ mgC/L}$$

7. Using the needle valve, adjust the flow of Nitrogen to 150 ml min^{-1} +/- 5 (with the old instrument we used a flow rate of ~15 ml/min). Check the nitrogen flow between the needle valve and the H_2SO_4 purge trap to obtain this flow rate using a bubble meter (40 seconds for 100 ml = 150 ml/min). Keep a record of the flow rate for each run.

8. Add 8 ml 6N H_2SO_4 to the purge trap. (Large purge trap = 10 ml, small purge trap = 6 ml – Amy added 8 ml in 0809). (Always turn the gas on before adding acid to the purge trap, and empty the acid before turning off the gas flow). Place a rubber serum vial cap over the

injection port on the purge trap. This setup will allow ~ 50 samples (14 DIC standards and 36 lake samples) to be analyzed in one batch, which is usually one lake.

9. Check the flow rate from the back of the Qubit by attaching a hose from the outflow on the Qubit to the bubble meter. The flow rate should be close to 150 ml/min. Keep a record of the flow rate for each run.

10. Adjust the zero knob on the Qubit to set the baseline reading to zero. If you are unable to obtain a reading of zero with the knob, you will need to adjust the coarse zero screw first. Find the middle of the zero knob setting, set the coarse zero to approximately zero, and then do the fine adjustment with the zero knob.

11. Start Peak Simple: Check settings and Start Data Acquisition (see below instructions for Peak Simple).

12. Zero Peak Simple Line (see below instructions for Peak Simple).

13. Using the 1000 µl Gastight syringe with a 1.5" 24 ga. needle, inject replicate samples of the DIC standard into the injection port on the purge trap (0.5, 0.7, 0.9, and 1.0 ml volumes) (Table 14). Similarly, use the 500 µl Gastight syringe to inject smaller replicate quantities (0.05, 0.1, 0.3 ml) (Table 1) (rinse syringe with next standard before injecting). **Note**: When withdrawing a volume of standard (or sample) from the serum vial be careful not to draw a vacuum or to incorporate air bubbles into the syringe, because they will affect DIC results. This will require some practice, especially when analyzing West Lobe Bonney. Always allow baseline to return to zero both on the Qubit and Peak Simple before injecting the next standard. Note the time of peak injections on Peak Simple so you can later match the peaks to their volumes.

14. Calculate the slope of the standard curve using these standards (peak areas can be obtained by going to "view" – "results" in Peak Simple). Keep a record of the slope for each run to check for instrument consistency.

Table 14. Volume of DIC standard used to create standard curve.

Lake	DIC Standard Injection volume (ml)	mg C
	1.00	0.060
BON FRX	0.90	0.054
BON HOR FRX	0.70	0.042
BON HOR FRX	0.50	0.030
BON HOR FRX	0.30	0.018
BON HOR FRX	0.10	0.006
BON	0.05	0.003

15. Once the initial set of standards are analyzed and the slope has been checked, begin analyzing replicate sample volumes based on the following Table 15 (rinse syringe with DIW followed by the next sample between samples). Sample volumes may be adjusted so that data output is within the range of the standard curve. If the replicate samples are not within 5 % of each other (this can be checked by going to "view" – "results" in Peak Simple), inject a third sample. Always allow baseline to return to zero both on the Qubit and Peak Simple before injecting the next sample. Note the time of peak injections on Peak Simple so you can later match the peaks to their sample names.

Table 15. Volume of DIC sample to inject for each depth and each lake.

West Bonney		**East Bonney**		**Hoare**		**Fryxell**	
Depth (m)	Injection Vol. (ml)	Depth (m)	Injection Vol. (ml)	Depth (m)	Injection Vol. (ml)	Depth (m)	Injection Vol. (ml)
4.5	0.7	4.5	0.8	4.5	1.0	4.5	0.6
5	0.7	5	0.8	5	1.0	5	0.6
6	0.7	6	0.8	6	0.5	6	0.5
8	0.5	8	0.8	8	0.5	7	0.2
10	0.5	10	0.8	10	0.2	8	0.2
12	0.3	12	0.3	12	0.2	9	0.1
13	0.2	13	0.1	14	0.2	10	0.1
14	0.05	15	0.1	16	0.2	11	0.1
15	0.05	18	0.1	18	0.2	12	0.1
17	0.05	20	0.1	20	0.2	15	0.05
20	0.05	22	0.1	22	0.2	18	0.05
22	0.05	25	0.1	25	0.2		
25	0.05	30	0.3	30	0.2		
30	0.05	35	0.4				
35	0.05	38	0.4				
38	0.05						

16. Once all the batch samples are analyzed, inject a single series of DIC standards based on Table 1. Combine these standards with the replicate standards run before the lake samples and create a standard curve. These post run standards will adjust for instrument drift that might have occurred during sample analysis.

17. Re-check the flow rate at both the start and end of the system as done above, and keep a record of these flow rates. Calculate the end slope for each run and compare to the start slope to check for instrument drift during the run.

18. Graph the Q-bit peak area output (see Peak Simple instructions below) versus the known concentration of the standards (Figure 2).

19. Use the standard curve regression equation to calculate DIC mg l^{-1} from the Q-bit output for each lake sample (Figure 7). The final reported DIC value is an average of the replicate samples.

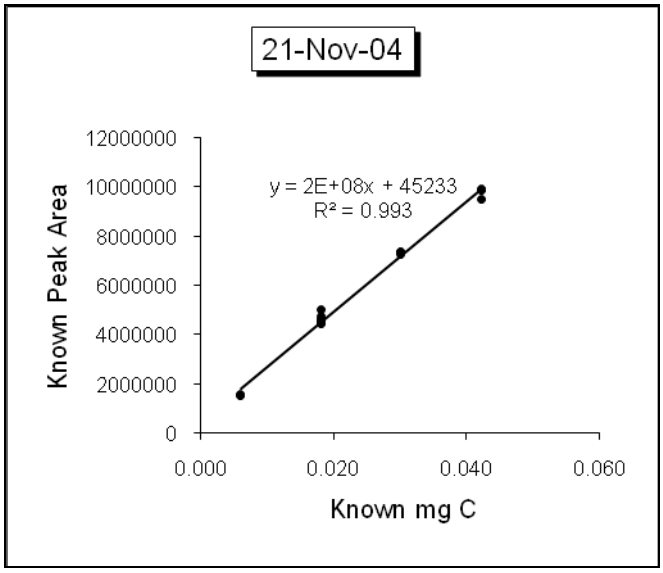


Figure 7. Example of regression equation used to calculate DIC from IRGA output.

$$\text{Sample mgC } l^{-1} = \frac{((\text{Sample Peak Area} - \text{Y Intercept})/\text{Slope}))*1000}{\text{ml injected}}$$

Peak Simple Instructions

Running Samples with Peak Simple:

- "Edit" – "Channels" – Channel 1 – "Details" – change "End time" to at least 180 min. "End time" is the amount of time the run will last. The default is 8 min, but you will most likely need at least a few hours, depending on how many samples you plan to run.
- "Edit" – "Channels" – Channel 1 – "Integration" – Here you can set your "area reject", which is the lowest area the program will integrate. You can leave it as default of 100. You can also access these setting in "View" – "Results" – "Integration."
- "View" – "Results" to get to peak area results. Unclick "Recognized Peaks Only" to view all peaks in file. "Close" to get back to chromatogram.
- "Acquisition" – "Run" – this will begin the active channel (1) running.
    - A blue line should appear along the baseline in the chromatogram, with a red integration line following it. Each time you inject a sample, the red line should follow and integrate the peak, signified by an open circle at the top of the peak. If very small changes in the baseline are being integrated, you can change the "area

reject" to a larger value so these changes are not integrated, and they don't show up in your results page. This probably won't be an issue.

- You can click the "auto-zero" button located to the left of the baseline on the chromatogram to adjust the line to zero. While the run is in progress, the baseline may drift a bit. You don't need to zero the baseline if the drift is small. As long as there are no leaks in the system, there should be no, or at least very little, drift.
- If the red line stops integrating during the run, continue the run. When the run is ended the integration will complete. During the run be sure to write down the time of each peak and the sample and volume that was injected.
- To zoom in or out on the chromatogram y-axis, press the + and – buttons to the left of the baseline.
- To adjust the x-axis time display, click the right or left arrows on the outside of either end of the timeline on the chromatogram.
- To scroll along the chromatogram time line, click the inside right or left arrows at the ends of the baseline.

- To view the integration results during the run, "View" – "Results" to get to peak area results.
- To stop the run at the end, "Acquisition" – "Stop."
- Go to "View" – "Results" to get to peak area results. Press "copy" and paste the results into an Excel worksheet. Close results.
- Save the chromatogram for later viewing by going to "File" – "Save As" – save as .CHR file. A .THU file will also be generated.

Peak Review & Manual Integration: For checking the integration of peaks after the run is finished.

- Press "ok" when the program says "Acquisition System not functioning." It is not hooked up to the system so it is ok.
- Open .chr file.
- To zoom in or out on the chromatogram y-axis and get the peaks in range, press the + and – buttons to the left of the baseline.
- To adjust the x-axis time display, click the right or left arrows on the outside of either end of the timeline on the chromatogram.
- To scroll along the chromatogram time line, click the inside right or left arrows at the ends of the baseline.
- "View" – "Results" to get to peak area results. Unclick "Recognized Peaks Only" to view all peaks in file. "Close" to get back to chromatogram.
- To manually integrate peaks where red integration line is not satisfactory, go to "Edit" – "Manual Integration." Click on the "Rubber Band" manual integration tool, which is the

button above the "undo" arrow on the left side of the screen. This took allows you to draw a straight line from left to right connecting any 2 points on the data line. Each time you manually integrate a peak, the program will change the area result in the "View" – "Results" page.

- If the baseline falls too low to zoom in on the peaks and see the base for manual integration, click on the "zero" manual integration tool (the bottom button on the left side of the screen), and click on the baseline to the left of where you want to "zero" the line. The "zero" tool will adjust the data line to the right of the selected point to the zero axis.
- To undo manual integration changes, click the "undo" button on the left of the screen. This will undo manual integration changes, but will not undo "zeros." If you want to start over, close the program, don't save changes, and open the file again.

Dissolved Organic Carbon by Total Organic Carbon Analyzer

General Discussion
Samples for Dissolved Organic Carbon (DOC) are run on the Shimadzu TOC-V series. Total Nitrogen (TN) is analyzed during DOC analysis using the Shimadzu TNM-1 analyzer. All samples must be acidified to pH 2 with 6N HCl in order to drive off all inorganic carbon. Be sure to check the sample pH with paper to ensure that the pH is around 2 – this is especially important for the bottom waters in Lake Fryxell and East Lobe Bonney.

Materials
Dry Valleys Lab
Graduated cylinder
125 ml amber borosilicate glass bottles (acid washed w/ 1% HCL: dump samples from previous year from bottles and remove tape, rinse 3X with hot tap water, rinse 1X with DIW, fill half-way with 1% HCL and cap with Teflon lined cap, let sit right-side up for at least 1 hour, flip upside down for at least 1 hour, rinse with DIW 3X. Remove caps and place foil loosely over top of bottle. Combust at 475° C for 4 h. Replace caps once bottles are cooled.)
Green TFE-lined caps (caps can be re-used from previous season if the glue is still holding the Teflon liner in the cap and the cap looks to be in good shape)
Bell Jar filtering apparatus with bottle stands
25 mm polysulfone filter funnels
25 mm GF/F filters (combusted at 475° C for 4 h)
Eppendorf repeater pipet and tips
Low range pH paper
MCM Crary Lab

Reagents
Dry Valleys Lab
Hydrochloric acid (HCL): 6N
MCM Crary Lab

Procedure
Below steps to be conducted in Dry Valleys Lab
Sample Collection
1. The filtrate produced from the chlorophyll-a filtration (Section 3.1) is collected for DOC analyses.

2. Gently invert the 1000 ml amber HDPE bottle, thoroughly mixing the sample, and decant 100 ml of sample into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the filter base, which is mounted on a vacuum bell jar, and replace tower. Place an acid-washed, combusted 125 ml amber borosilicate glass bottle

directly under the filter base. The filter is used as a replicate for Chlorophyll-*a* analysis (see Chlorophyll-*a* method); therefore, filtering must be performed in the dark.

4. Filter sample under low vacuum (< 7 in Hg). Collect 100 ml of the filtrate in the 125 ml glass bottle. Do not rinse the filter tower with DIW if you still need to filter the nutrient sample. Only rinse the filter towers between sample depths. Carefully remove the bell tower and cap the amber bottle. DOC samples can be kept on the floor of the lab during the filtration process, or placed at 4° C.

5. Once all of the DOC samples are filtered, preserve each 100 ml sample with 1.0 ml of 6N hydrochloric acid using the Eppendorf repeater pipet. If less sample volume is filtered, adjust the volume of hydrochloric acid accordingly. Check the sample pH with paper to ensure that the pH is around 2 – this is especially important for the bottom waters in Lake Fryxell and East Lobe Bonney. More HCL may need to be added to these samples to reach a low enough pH for analysis. Store the samples in the dark at 4° C until analysis.

Below steps to be conducted in MCM Crary Lab

The following is the old method – the current method needs to be entered – for current method see - http://www.mcmlter.org/restricted/instruments/shimadzu/Shimdadzu.htm

Sample Analysis

1. Open precleaned ampoules.

2. Add 2ml of sample to ampoule.

3. Add 200 µl of 5% HCL (prepared with Organic free water) to ampule.

4. Purge using air on Purging and Sealing unit for approx. 5 minutes.

5. While maintaining purge, add 1 ml of 100 g/l Sodium Persulfate solution (prepared as directed by TOC manual and purged with nitrogen) to ampoule. Do this step individually just before sealing.

6. While maintaining positive oxygen flow in the ampoule, seal the ampoule.

7. Bake the sealed ampoules at 105 °C for about 8 h to drive the reaction to completion.

8. Prepare a standard curve from the stock organic carbon solution (10 – 50 µg C) and a blank.

9. Analyze samples and standard curve with the TOC analyzer (Consult the TOC manual for injection procedures).

10. Use the regression equation of the standard curve and calculate the DOC (mg/l) for each sample.

Notes:

1. Samples with greater than 50 μg C must be reanalyzed with an appropriately reduced sample volume.

2. Some samples will have high carbonates and fizz when acid is added. Add acid slowly so as not to loose sample.

3. It is important to maintain a pure oxygen atmosphere inside the ampoule. This will ensure that atmospheric CO_2 does not intrude, and will also aid in complete oxidation of the organic carbon.

4. Do not allow the sample to sit for long periods of time between procedure steps 10 and 11, as oxidation of carbon within the sample will begin and some carbon will be lost as CO_2.

Particulate Carbon and Nitrogen Analysis by Elemental Analyzer (Filters and Sediment)

General Discussion

General Discussion

This method is used in the analysis of carbon and nitrogen on filters (water) and sediment samples that have been prepared using ASA Analytical Services method described below. Samples are analyzed with a CE Instruments Flash EA 1112 elemental analyzer which flash combusts at 1800° C. Combustion gases pass through a catalyst converting all carbon and nitrogen combustion products to CO_2 and N_2. The gases are then separated by gas chromatography and detected by a thermal conductivity detector.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

Graduated cylinder
25 mm GF/F filters (combusted at 475° C for 4 h spread out on aluminum foil that will serve as a wrapper after combustion)
25 mm Polysulfone filter towers
Vacuum pump
Filter forceps
Aluminum weigh boats
Zip Lock Bags

MCM Crary Lab

Elemental analyzer: ThermoQuest EA 1112 Flash
UHP Helium (GC Grade) (carrier gas)
Oxygen 99.995% (for oxygen inject)
Forceps: Assorted sizes and types
Glass plate
Metric ruler
Micro spatula
Micro-balance
Pre-formed tin cups (ThermoFisher part # 252 080 00)

Interferences

1. The elemental analyzer is very sensitive and will detect organic carbon and nitrogen in fingerprints. Keep bench area very clean, wear gloves; handle all samples and standards with forceps to avoid contamination.

2. Ensure that the preparation area is clean of any standard when working with samples.

Reagents

Standards:

Acetanilide: standard grade, pre-dried at 85 °C and desiccated (% C = 71.09, % N = 10.36).
Soil reference standard: pre-dried at 85 °C and desiccated (% C = 3.5, % N = 0.37).

Combustion and reduction reagents:

Oxidation catalyst: ThermoFisher product # 338-400-60
High Quality Copper: ThermoFisher product # 338-353-12

Procedure

Sample Collection

1. Fill a 1000 ml HDPE amber bottle (rinse 3x with sample water before filling with sample) with 1000 ml of sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport.

Below steps to be conducted in Dry Valleys Lab

2. Particulate organic carbon and nitrogen lake water samples are taken from the 1000 ml amber Nalgene bottle. Gently invert the bottle, thoroughly mixing sample, and decant 500 ml into a graduated cylinder.

3. Use a six place manifold with 25 mm polysulfone filter towers. Place a combusted 25 mm GF/F onto the polysufone filter base, replace tower, and filter the sample under low pressure (<10 in Hg). **Note**: The filter tower will only hold 200 ml of sample, therefore, continually top off the sample until the entire volume is filtered. Furthermore, many of the Lake Bonney samples will take between 6-12 h to filter, thus plan accordingly. The entire volume should be filtered, even for chemocline depths at Lake Bonney and Lake Fryxell. Record the volume of water filtered for each depth in each lake.

4. Once the entire volume is filtered, do not wash the filters. Just suck them dry unless there is a large particle load sticking to the filter tower (such as may occur when filtering muddy stream water). (Prior to the 0405 season, it is possible that after filtering, the filters were rinsed, while still in the filter tower, with approximately 20 ml of *DI* to remove salts. This was not done between 0405 and 1011, as it was only stated in the Appendix of the manual and was not included in the main method, nor was that techniques passed on to Amy during training. After discussion with Kathy and John, the above method was decided on). Remove filter with forceps and place (organic matter up) in an aluminum weigh boat with the sample information (including volume filtered) clearly etched into the bottom of the dish. Dry at room temperature for 12 h (dry weigh boats on clean aluminum foil). Make sure to keep the bottom of each weigh boat clean as the bottom of each boat will be in contact with the filter in the boat under it during packaging. Following desiccation, stack all of the aluminum weigh boats together, ensuring the bottoms are clean. Place an empty one on top. Tape together, wrap in foil, and store frozen in a zip lock bag. **Include a GF/F filter from the same packet of combusted filters used for the samples to be analyzed as a blank.**

Below steps to be conducted in MCM Crary or MSU Lab

Sample preparation

Both filter and sediment samples must be treated with acid to remove inorganic carbon, which occur as carbonate. Fuming HCL converts inorganic carbonate in samples to water vapor, CO2, and Calcium Chloride (Grasshoff, K., M. Ehrhardt and K. Kremling (eds). 1983. Methods of Seawater Analysis. Verlag Chemie).

1. Place two 50 ml beakers with ~40 mls of concentrated (12N) fuming HCL, and one 50 ml beaker with DIW (to increase humidity), into the center of a glass desiccator (without desiccant) or a similar covered container of sufficient size to allow for the addition of samples. A glass Pyrex rectangular dish with a second glass Pyrex rectangular dish upside down on top, sealed together with high vacuum silicone grease on the edges, works well for this (Figure 1). It is advised to use fresh HCL each season and to replace the beakers in the acidifying container after two acidification runs.

2. Place filters onto the top of clean, uncovered scintillation vials inside the container (Figure 1), and allow to react for 24-48 hrs (keep aluminum weigh dishes once filters have been removed for use later). Soil samples can be placed directly into clean vials, however ensure that the soil is spread as close to a monolayer as possible (fumes may not diffuse through thick soil volumes). Note that the vials cannot be labeled because of the acid fumes (the fumes will eat away the label), so be sure to note the order of the vials when placing them in the container. Place an aluminum tab from a weigh dish inside the container to verify that the acid fumes are strong enough (the aluminum tab should turn white and fluffy from the acid fumes). If the tab does not turn white and fluffy, replace the HCL in the beaker, or, if your HCL was not fresh, obtain fresh HCL.

3. Following acidification, place filters back in their aluminum weigh dishes and place in drying over at 90ºC for 4-12 hours.

Figure 1. Acidification of filters.

Instrument Preparation (prior to each season)

- In the Flash EA 1112 Instrument Operating Manual we use the "NC-soils, NC-Sediments, NC-Filters Determinations" method. There is also a Software Manual and a Parts Manual.

- It is important to wear gloves when handling and packing the quartz column. Fingerprints can cause the tube to crack or shatter causing injury.

- Use the diagram in Figure 6 (this can be found on page 75 of the operating manual for "Analyzer set-up for NC-soils, NC-Sediments, NC-Filters") as a guide to setting up reactor 1, reactor 2, and the adsorption filter.

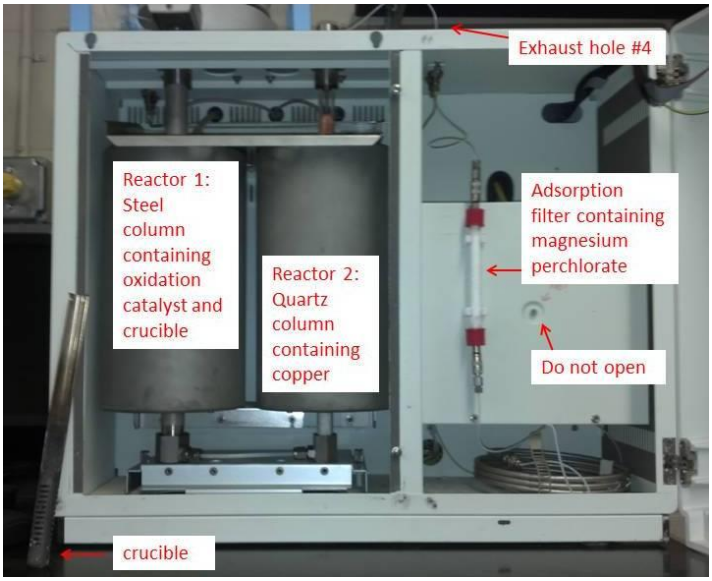


Figure 2. CE Instruments Flash EA 1112 elemental analyzer.

Auto Sampler: It is best to check the auto sampler for problems at the start of each season. Remove the auto sampler by removing the 2 screws on top and 1 in front (Figure 3b). When replacing the screws, be sure to only tighten hand tight! The viewing window in the auto sampler can be replaced if it cracks. The o-ring under the viewing window can also be cleaned or replaced. Clean the auto sampler piston (Figure 3a) once per year or as needed for trouble shooting. Software is needed to eject the piston. In the Eager 300 software, click on Tools → "Cleaning MAS Piston." Click on "step sampler tray piston" and the piston will eject from the front of the auto sampler. Clean the o-rings on the piston and put a light coating of high vacuum grease over o-rings. If the o-rings need to be replaced, you will need the brass tool (Figure 3c). Note that o-rings are directional and have 1 tapered end. When running samples, keep the plastic cover on top of the auto sampler to keep the air out.


Figure 3a.

Figure 3b.

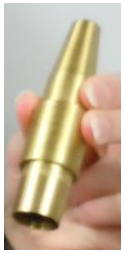


Figure 3c.

Reactor 1 – HPAR reactor (steel column): HPAR reactor (combustion column). This must be changed each season. Remove the column from the left side of the CN analyzer (Figure 2, 5a), pull out the old wool from bottom and use the larger plunger tool (Figure 4) to push out the old oxidation catalyst (this will take some effort!). Place quartz wool (use quartz wool in the hood because the fibers are an irritant to lungs) at the bottom of the column and gently pack down with the smaller plunger tool (Figure 4) (try to keep the long quartz wool fibers together when packing; i.e., don't use small broken pieces of quartz wool but instead long, intact pieces) to at least 50 mm (Figure 6). Fill with oxidation catalyst (60 g, ThermoFisher product # 338-400-60) by opening the serum vial containing the oxidation catalyst (Figure 5b), putting in a beaker and using a spatula to evenly distribute the two types of catalyst into the column (Figure 5c). The catalyst comes in 60 ml serum vials with a pre-measured amount to fill the column to the correct height. Place quartz wool in the top of the column above the catalyst and gently pack down with plunger tool. Put enough quartz wool so that when inserted, the crucible sits even with the top of the column (this will be more than the 10mm specified in Figure 6). Insert the packed column into the instrument from the top of the instrument. Grasp the bottom of the column and gently push into the reactor bottom. While still holding onto the column, place the O-ring onto the top of the tube. Seat the auto sampler onto the O-ring and screw down hand-tight. **Note**: The combustion tube catalysts have a limited sample life. When CCV's start to approach the lower limits of acceptable range, or the tube is full, a new tube must be prepared.

Figure 4.

Figure 5 a, b, c.

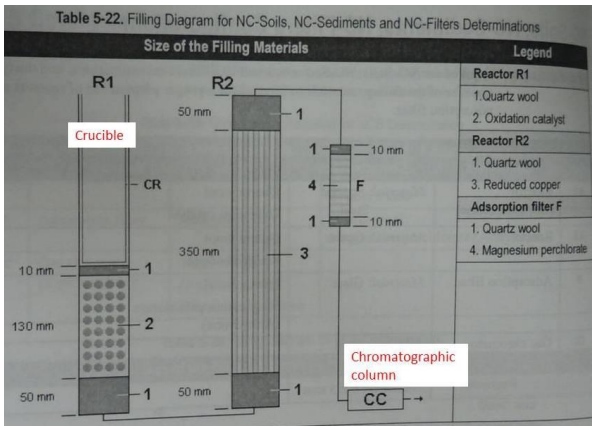


Figure 6. Filling diagram for NC-Soils, NC-Sediments and NC-Filters.

Reactor Bottom Caps: The reactor bottom caps (Figure 7) will get corroded over time and will need to be cleaned. Replace the o-rings (use Bottom o-rings (25mm and 18mm)) and lightly grease with high vacuum grease once a season or as needed for troubleshooting. Clean out the channel in which the o-ring sits with a spatula and Kimwipe. Scrape corrosion on the inside where the piston sits with a spatula and Kimwipe.


Figure 7.

Reactor Top Caps: Replace the o-rings in the reactor top caps (use Top o-rings) and lightly grease with high vacuum grease once a season or as needed to troubleshoot. The o-ring for reactor 1 is orange; the o-ring for reactor 2 is black (shown in Figure 8). Wipe out the top caps with a Kimwipe.


Figure 8.

Instrument Preparation (prior to each run)

The crucible and adsorption filter can be changed with the instrument in stand-by mode if you don't want to shut the instrument completely off between sample runs; if you need to remove the quartz column the instrument should be shut off and allowed to cool.

Crucible: The crucible must be cleaned before each run. To remove, take off the auto sampler and pull the crucible out of reactor 1 using an allen wrench to hook the holes in the top of the crucible (it will be very hot if you have just finished a run!). Clean the crucible using the smaller plunger tool, taking care to clean out the vents (Figure 9). Pack a plug of quartz wool in bottom of the crucible (use quartz wool in the hood because the fibers are an irritant to lungs) using the smaller plunger tool, making sure not to pack the quartz wool higher than vents. Replace the crucible in reactor 1.

Figure 9.

Reactor 2 – Quartz reactor (quartz column): Remove the column from the right side of the CN analyzer (Figure 2, 10). DO NOT TOUCH THE COLUMN WITH BARE HANDS – WEAR GLOVES. When gray copper in the quartz column fills 2/3 of the height of the furnace chamber (Figure 10c – in this figure the furnace height is only ½ gray), (~150 samples), change the column. The unused copper can be put back at the top of the column once the bottom is filled with new copper. Clean the old quartz column or use a new one (use each column only twice as the glass will get brittle after being used and cleaned). Pack the bottom of the new (or cleaned) column with at least 50 mm of quartz wool (use quartz wool in the hood because the fibers are an irritant to lungs). Fill the column with High Quality Copper from approximately two 50g ampoules (ThermoFisher product # 338-353-12) by shaking it into the column (the shaking also helps decrease air spaces in the column) to a height as in Figure 10a and 10b. See also Figure 6. Extra copper that is not used can be saved in a serum vial and stored in the dessicator. Tap the column on a bench to settle the copper pieces in the column. Put a plug of quartz wool on top of the copper. Insert the packed column into the instrument (the bottom of the column is the beveled end) from the top of the instrument, being careful not to chip the end of the column on the inside of the furnace. Grasp the bottom of the column and gently push into the reactor bottom. While still holding onto the column, place the O-ring onto the top of the tube. Seat the top cap of reactor 2 onto the O-ring and screw down hand-tight.



Figure 10 a, b, c.

Adsorption filter (glass): This is a moisture trap. Put quartz wool in the ends and fill with magnesium perchlorate (Anhydrone) (Figure 2, 6). Change every 2-3 runs or as needed. The red end caps contain Teflon liners that can be replaced. Quartz wool can be re-used when replacing mg perchlorate. Use quartz wool in the hood because the fibers are an irritant to lungs.

Once the instrument is ready, proceed with instrument set-up and perform a leak check to ensure a helium tight seal.

Instrument set up

1. Open the valves to the oxygen and helium cylinders. Set the gas pressure on the regulators to 36.25 psi for Helium and 36.25 – 43.5 psi for Oxygen.

2. Turn the main instrument power switch on (located in the lower back right of the instrument). Remove vent plug (the brass screw pointed to by the pliers in Figure 8) before turning instrument on.

   If reactor 1 has been changed, you will need to run the system (turn on the temperature and flow (see below); the detector does not need to be on for this) with the top cap of reactor two removed to vent out moisture in reactor 1. You will see moisture in the connector tube between the bottom of reactor 1 and 2. It will take approximately 2 hours for the moisture to disappear. You can also look for moisture at the top of the copper column. Note that once the system is running, it will take 30-45 samples to condition the system with the new reactor.

3. Turn on the instrument computer and boot up the Eager 300 software package "Eager 300 for EA H12."

4. Click "Eager 300 for EA1112" → click on "EA1112 #1."

The instrument parameters should be set as below, according to the instrument operating manual. Once set, they should not have to be changed.

a. Temperature
   i. Left Furnace: 900 C
   ii. Right Furnace: 700 C
   iii. Oven: 50 C
b. Flow/Timing
   iv. Carrier: 140 ml/min
   v. Oxygen: 225 ml/min
   vi. Reference: 100 ml/min

5. To set the parameters, click on "Edit Elemental Analyzer Parameters" (Figure 11) and set the Temperature (Figure 12) and Flow/Timing (Figure 13) as above. Click "Send" on the Temperature and Flow/Timing tabs, once you have set the parameters, to turn the Temperature and Flow in the system on. After you have entered the values, if you click no on the "save option" it will cause you to enter the values again before the next run.

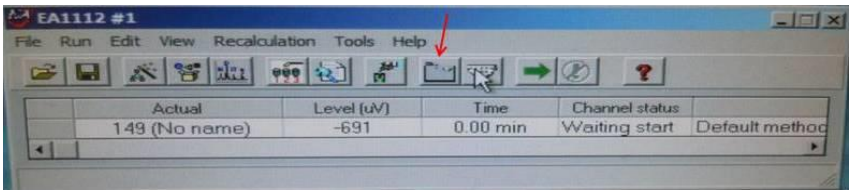


Figure 11.

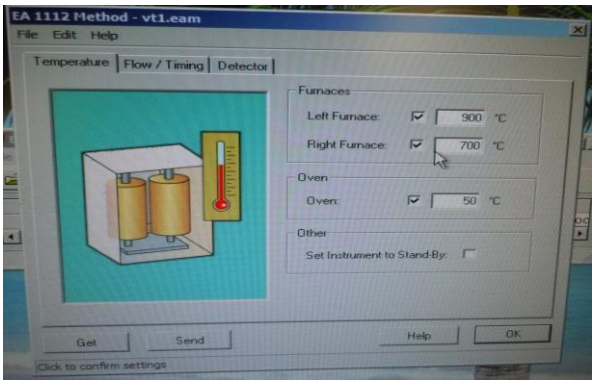


Figure 12.

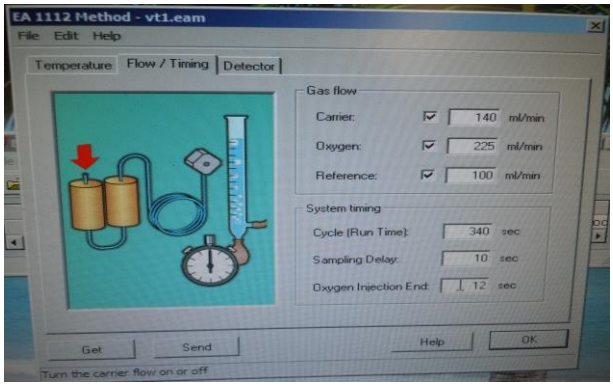


Figure 13.

6. Click "View Elemental Analyzer Status" to see when the system is up to temperature (Figure 15, 16).

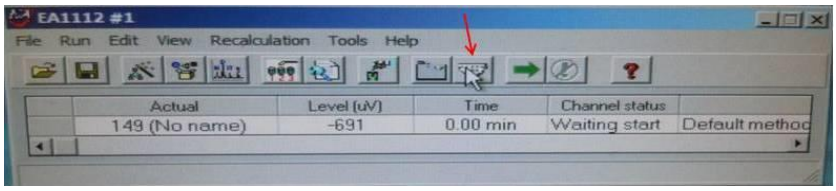


Figure 15.

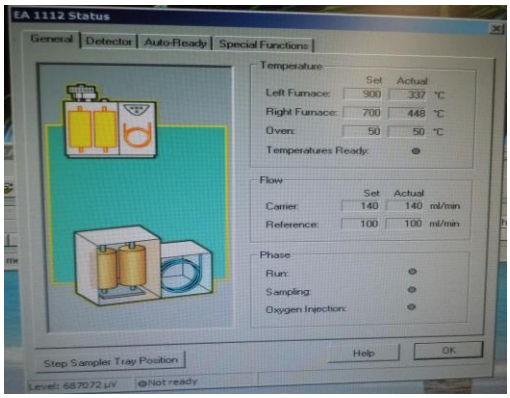


Figure 16.

7. Click "Send" in the Detector tab (Figure 14) to turn on the detector if you are ready to run samples.

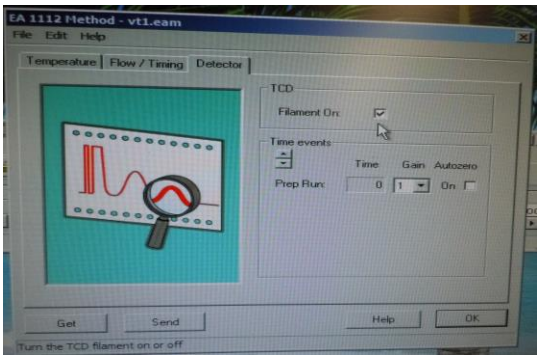


Figure 14.

8. **Perform a leak test:** A Helium tight seal is indicated by a carrier flow reading < 5 ml/min @ 360 seconds, as per CE's "official leak test procedure."

    a. Click "View Elemental Analyzer Status" → "Special Functions" → "Leak Test." Don't check the boxes → Click Start, click Yes (Figure 15). The Carrier Flow should be <5 ml/min before 360 seconds. Press Stop when it reaches the target value or time (usually the flow will drop to 1 ml/min if you wait). Click Done. If the system does not pass the leak test, try and isolate the leak by isolating different sections of the system. The exhaust from the instrument comes from hole #4 on top of the instrument (Figure 2). It can be plugged to troubleshoot leaks. During the run it should remain open. It should be plugged when not in use.

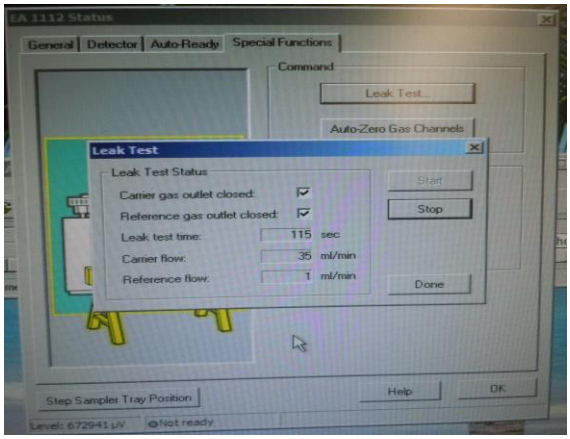


Figure 15.

9. **Reset Maintenance Program:** Click on "View" in main screen → "Maintenance" → "Edit - Reset Maintenance program." Left 1 = crucible – reset; left 2 = oxidation catalyst - reset; right = copper – reset. Reset each of these when they have been replaced. This will remind you of when maintenance is due based on the number of samples run. The times for maintenance in the program have been set by manually by our instrument technician.

10. **Auto-adjust the baseline:** Click "View Elemental Analyzer Status" → "Detector" and auto-adjust at 1000 mv. At the end of a run the baseline may have dropped to 980 mv - this is ok.

Instrument Calibration

It is a good idea to run a standard curve before running your samples to ensure the system is working properly, but standards will also be run with every sample sequence and combined from all the sample runs to create a master calibration curve for the season. Below is an outline for running the standard curve.

1. Calibration standards are made in tin cups (ThermoFisher part # 252 080 00). Turn on the micro-balance and let it warm up for ~ 10 minutes. The standards (acetanilide and soil reference) should be dried once per week at 85° C. At the start of the season, dry the standards for ~4 hours, then for 1 hour each week. Standards should be put in the desiccator with the cap off for a few minutes after drying in the oven. (Check the temperature of the drying oven at MSU with a thermometer. Hold arrows for 5 seconds to set temperature).

2. Clean items that will be used for packing the tin cups (glass plate, forceps, micro-spatula, crimper and cylindrical tool (Figure 16)) with ethanol, wipe well with a Kimwipe, and wipe down with DIW.

Figure 16.

3. Using forceps, place a single tin cup on the micro-balance and tare. Remove the cup from the balance and place on the glass plate.

4. Use the micro-spatula to add a small amount of acetanilide standard to the tin cup. Record the weight of the tin cup containing standard. Place the tin cup in the crimper and use the cylindrical tool to fold the top of the cup over, crimp and compact. Weigh again to ensure no standard was lost during crimping. Prepare a range of acetanilide standards, making a total of at least six standards, ensuring the weights are spread out evenly between 25 and 1000 µg.

5. Weigh between 100-200 mg of soil reference standard following the procedure for the acetanilide standards. When done with the micro-balance, press the brake and shut off.

6. Crimp three empty tin cups to be used as blanks.

7. Load the auto sampler rack, starting with three instrument blanks (empty places in the auto sampler), the three tin cup blanks, and ending with the high standard. Position the rack to the zero place BEFORE filling the rack.

8. Set up the Sample Table in the Eager 300 Software by clicking on the "Sample Table" icon as shown in Figure 16. At the end of the previous sample table (any samples previously run will still be saved in the sample table): click on "Edit sample" → "Fill sample table."
    i. Sample name: No name.
    ii. File name = Enter date.
    iii. No. samples = Enter the number of samples you have in the auto sampler, including zero.
    iv. Sample name index = 0
    v. File name index = 001
    vi. Click "Add" and enter sample names manually.
    vii. Click column A on the row of your first sample to set the program to the actual sample that will be acquired once you start the instrument. This will set the program to record data for the correct sample once you start the run.

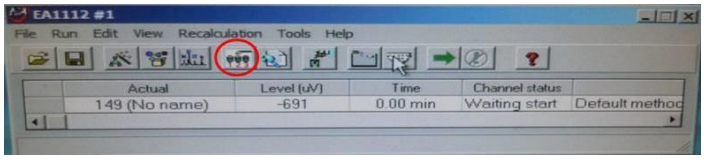


Figure 17.

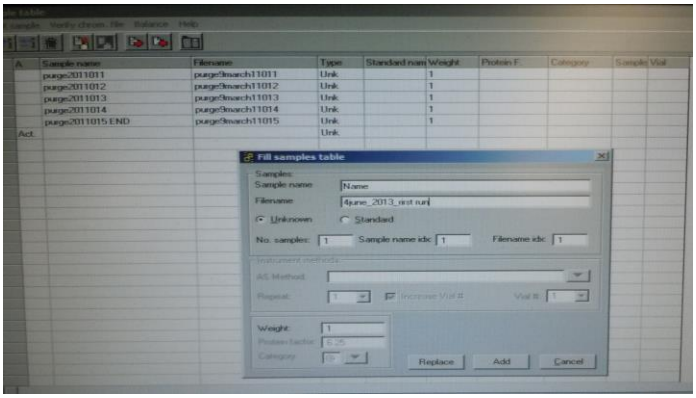


Figure 18.

9. Put the auto sampler containing samples on the CN analyzer.

10. Reset the baseline with autoadjust if it has drifted.

11. Start the run by pressing the green arrow on the main screen "Start sequence of samples."

12. Choose the conditions you want when the sequence of samples is finished and press "Start now." You can choose to have the instrument go into Standby mode (choose this option if you plan to run a second set of samples after the first set is finished), or to have the instrument shut down once it finishes the sample run.

13. If you have a lot of samples to run and the auto sample tray is full, it is useful to have 10 or so "purge" samples listed at the top of the sample table. These can be used while you are setting up your sample run to purge the instrument of air before you start your samples. This way you won't need to use auto sampler places in the tray containing your samples for purging during your sample run. Place an empty auto sampler tray on the instrument and set the instrument to run the purge samples from the sample table. Once you are ready to start your samples, press stop to stop the purge run, replace the auto sampler tray with the one containing your samples, and start the run over on the appropriate sample in the sample table.

14. Determine the theoretical yield of carbon and nitrogen for each standard. Acetanilide contains 71.09% C and 10.36% N. Multiply each standard weight by the fraction of C and N in the standards to determine the expected μg of each C and N, respectively. For example, 25 μg acetanilide contains 17.77 μg C and 2.59 μg N; 550 μg acetanilide contains 390.99 μg C and 56.98 μg N.

15. Subtract the average area of the tin cup (determined from the tin cup blanks in step #6) from each standard area. Plot the peak area (see *Data Processing* section below to learn how to determine the peak area of your standards) vs. the expected μg for each standard and ensure a linear fit with a correlation of 0.995 or better, to validate calibration (note that one curve will be made for carbon and one curve for nitrogen) (Figure 19). Instrument blanks do not need to be included in the standard curve. Because the CN analyzer is very stable, standards run with

each set of samples can be added to this curve and the resulting master curve (C or N) can be used to determine the mass of carbon or nitrogen in each sample (see *CN Calculations* section below).

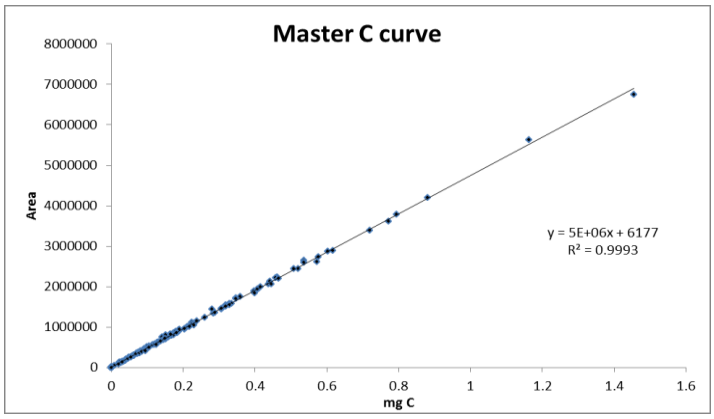


Figure 19.

Sample Analysis (acid-treated filters):

1. Using forceps (clean as described above), place sample filter on a clean glass plate.

2. Using forceps, fold the filter onto itself twice, and place into a tin cup that has been inserted into the crimper. (More detailed instructions can be found in the Flash EA 1112 Operating Manual, page 121). Be careful not to puncture holes in the tin cup. Crimp and compact the tin cup over to completely cover the sample filter using the cylindrical too (Figure 19).

Note: This step is largely technique based and, if possible, should be demonstrated by an experienced analyst.

3. Prepare at least 6 acetanilide standards, a soil reference standard and several tin cup blanks as described above in the *Instrument Calibration* section.

4. Place the encapsulated samples into the cells of the autosampler tray, dispersed with the acetanilide standards, the soil reference standard, the tin cup blanks, filter blanks and instrument blanks (see *Quality Control* section below). Enter the samples into the sample table of the Eager 300 program, and start the run as describe in the *Instrument Calibration* section. The acetanilide standards from all sample runs will be combined to create a master curve for Carbon and a master curve for Nitrogen (Figure 19).

Sample Analysis (sediment):

1. Ensure that sediment samples are as homogeneous as possible. Handle all sample cups with forceps to prevent contamination from your skin.

2. Weigh out 15-250 mg of dried sediment (105 °C for >24 h) into a tared, pre-formed tin cup. The same tools are used for sediment samples as outlined above for filters.

Note: This step is largely technique based and, if possible, should be demonstrated by an experienced analyst.

3. Follow the same method as describe above for filters for setting up the sample run.

Quality Control

1. Analyze one blank cup per 20 samples. Ensure that the blank measures at or below the instrument detection limit to check for instrument drift.

2. Analyze one mid-range standard per 20 samples. Ensure a percent recovery between 90 - 110 %, when compared to the standard curve, to continue analysis. If the recovery is not within this range, check instrument operation.

3. Analyze one duplicate sample per 20 samples. Ensure a coefficient of variation of between 70 - 130 % to confirm precision.

Instrument Shut Down

1. In the software, click "shut off detector and gas." Turn the instrument to stand-by and wait for the combustion chamber to reach stand-by temperature.

2. Turn the gas flow at the gas cylinders off.

3. Turn the main power to the instrument off.

4. Replace vent plug (brass plug in Figure 8) when completed and instrument is off to keep dust out of the instrument. Also be sure to replace the plexiglass cover over the autosampler when the instrument is stored for a while.

Data Processing

1. Open Eager 300 for EA 1112
    a. Click on "View" → "View chromatograms."
    b. Click on "File" → "Load chromatogram" and choose the file you want to view based on the date and number of sample as it was run (0 spot on autosampler is sample #1). The file name will be as you named it but you can't see this until you open the file (Figure 20).
    c. Set the manual scale to look at the chromatogram: Click on "View" → "Set manual zoom" and set: Full Scale V = 10; Scale offset = 0; Start time = 0; end time = 5.67 (this is how long it takes to run each sample).
    d. The computer program quantifies the carbon peaks; you may need to quantify the N peaks manually.

e. Click and drag the box to zoom in on the N or C peak. For the N peak, zoom in to view the peak at ~1.7 mvolt scale to look at peak (it is best to be consistent with the scale between samples if you need to manually integrate the peak). Retention times: N = 1.8; C = 3.6.
f. Manual integration: To manually integrate a peak click on "Peak" → "Add peak." Hold the cursor above the baseline where you think the peak starts and a red line will appear on the baseline. Click where you want peak to start, and a dotted line will appear. Drag the cursor to where want the peak to end and click there to end integration. It is best to manually set the window to be the same every time when manually integrating peaks (i.e. 0-1.7mV on y-axis and 1.2 to 2.4 mins on the x-axis for a nitrogen peak).
g. When you have adjusted the peaks as desired, click on "peak" → "show peak data" and write down the area values. The arrow at the bottom of the chromatogram tells you which peak you are viewing. When zoomed out the arrow is on the last peak you were looking at.
h. If want to start over, click on the "Home" button (Figure 20).

Figure 20.

CN calculations:

The mass of carbon and nitrogen in each sample is calculated from the master standard curve (described in the *Instrument Calibration* section above) using the peak area for each sample (Figure 19). The final concentration in the lakewater is calculated as follows:

$$\text{Carbon } (\mu g/l) = \frac{(((\text{sample area} - \text{blank area}) - \text{y-intercept})/\text{slope}) * 1000 * 1000}{\text{ml filtered}}$$

Where:
sample area = area determined by the CN analyzer (sample + filter + tin cup)
blank area = average area of blank filters determined by the CN analyzer (filter + tin cup)
y-intercept = area when Carbon or Nitrogen mass is zero
slope = area/mg C or N

1000 = conversion factor (µg / mg)
1000 = conversion factor (ml / L)
ml filtered = ml of sample filtered in the field

References

CE Instruments Flash 1112 Elemental Analyzer Operating Manual.
CE Instruments Eager 300 Software Operating Manual.
CE Instruments Flash EA 1112 Consumables and Spare Parts Catalog.

CAHN micro balance – 20 mg standard weight = 19.900 mg, 10 mg standard weight = 10.023 mg.

Total Particulate Phosphorus Analysis: Manual Method

(This analysis was started during the 2003-2004 season)

General Discussion

This method for the analysis of total particulate phosphorus in natural waters is based on the digestion procedure to Solorzano and Sharp (1980), and on a modification developed by Murphy and Riley (1962). Residue collected on a filter is baked at high temperature to decompose organic phosphate. Polyphosphates are then acid hydrolyzed to yield orthophosphate. Analysis of orthophosphate depends on the reduction of a stable phosphomolybdate complex by ascorbic acid in the presence of antimony. A blue sol is produced, proportional in color intensity to orthophosphate concentration within the range 5-250 $\mu g\ P\ l^{-1}$.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

Graduated cylinder
Vacuum pump
25-mm GF/F filters, pre-combusted (to remove organic phosphorus) and acidified. (Combustion protocol: combust at 475°C for 4 hrs spread out on aluminum foil that will serve as a wrapper after combustion and acidification. Acidification protocol: soak in 1% HCL for 3-4 hours, rinse 4X with DIW, lay out on combusted foil to dry, and wrap in foil once dried).
25 mm Polysulfone filter towers
Aluminum weight boats
Zip Lock Bags
Filter forceps

MCM Crary Lab

Aluminum Foil
Glass scintillation vials (20 ml vials with HDPE cone caps; acid rinsed)
Plastic-lined Caps (acid rinsed)
Test Tubes (Acid rinsed)

Reagents

Acid molybdate-antimony solution: Add 7.5 g ammonium paramolybdate $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ to 500 ml *DI* water. Add 0.14 g antimony potassium tartrate to 88 ml concentrated H_2SO_4. Combine solutions and vortex, allow to cool, then dilute to 1000 ml with *DI* water. Store in a dark glass bottle (stable for months).

Ascorbic acid: Dissolve 2.5 g L-ascorbic acid in 100 ml *DI* water. Prepare daily.

Hydrochloric acid (0.2 M HCl): Dissolve 16 ml of concentrated HCl 1000 ml *DI* water. Store in a glass bottle (Stable for months).

Magnesium sulfate solution (0.017 M $MgSO_4$): Dissolve 2 g $MgSO_4$ (or 4.2 g $MgSO_4\ 7H_2O$) in 1000 ml *DI* water. Store in a glass bottle (Stable for months).

Mixed reagent: Mix 4 parts of acid molybdate-antimony solution with 1 part ascorbic acid solution. Prepare daily.

Sodium sulfate solution (0.17 M Na_2SO_4): Dissolve 12 g anhydrous Na_2SO_4 in 500 ml DIW. Store in a glass bottle (Stable for months).

Procedure

Sample Collection

1. Fill a 1000 ml HDPE amber bottle (rinse 3x with sample water before filling with sample) with 1000 ml of sample from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times). Place in cooler for transport.

Below steps to be conducted in Dry Valleys Lab

2. Particulate phosphorus lake water samples are taken from the 1000 ml amber Nalgene bottle. Gently invert the bottle, thoroughly mixing sample, and decant 500 ml into a graduated cylinder.

4. Use a six place manifold with 25 mm polysulfone filter towers. Place an acid leached 25 mm GF/F onto the polysufone filter base, replace tower, and filter the sample under low pressure (<10 in Hg). **Note**: The filter tower will only hold 200 ml of sample, therefore, continually top off the sample until the entire volume is filtered. Furthermore, many of the Lake Bonney samples will take between 6-12 h to filter, thus plan accordingly. The entire volume should be filtered, even for chemocline depths at Lake Bonney and Lake Fryxell.

♦♦♦♦♦ Record the volume of water filtered for each depth in each lake. ♦♦♦♦♦

4. Once the entire volume is filtered, remove with forceps and place the filter (organic matter up) in an aluminum weigh boat with the sample information (including volume filtered) clearly etched into the bottom of the dish. Store weigh boats on clean aluminum foil while the rest of the samples are filtered. Make sure to keep the bottom of each weigh boat clean as the bottom of each boat will be in contact with the filter in the boat under it during packaging. Once all the samples are filtered, stack the aluminum weigh boats together, ensuring the bottoms are clean. Place an empty one on top. Tape together, wrap in foil, and store frozen in a zip lock bag.

Below steps to be conducted in MCM Crary Lab
(this is not the method used – the current method write-up is being finalized)

5. Rinse filter twice with 2-ml aliquots of 0.17 M Na_2SO_4.

6. Transfer filter to an acid-rinsed 20-ml scintillation vial and add 2 ml 0.017 M $MgSO_4$.

7. Dry the sample completely in a clean oven at 95° C.

8. Loosely cover the bottle with aluminum foil. Transfer the bottle to a muffle furnace and bake at 450-500° C for 2 h.

9. After cooling, add 5 ml of 0.2 M HCl and cap (acid-rinsed polypropylene-lined).

10. Heat sample in 80° C oven for 30 min.

11. After cooling, pour supernatant into an acid-rinsed test tube. Rinse bottle with 5 ml *DI* water and add rinse to test tube.

12. Add 2 ml of mixed reagent and vortex thoroughly.

13. After 10 min, read absorbance at 885 nm.

Preparation of Standards:

1. For each standard, add 0.3 ml 0.17 M Na_2SO_4, 2 ml 0.017 M $MgSO_4$, 5 ml 0.2 M HCl, and 2.7 ml *DI* water to an acid-rinsed test tube.

2. Add PO_4^{3-} standard solution to each tube, as appropriate. (e.g. the addition of 50 µl of 50 µg P l^{-1} standard solution yields a standard concentration of 248.8 µg P l^{-1}).

3. Add 2 ml of mixed reagent, vortex, and read absorbance as for samples

Ammonium Analysis by Autoanalyzer

General Discussion

Ammonia is reacted with alkaline phenol followed by sodium hypochlorite, forming indophenol blue. Sodium nitroprusside is added to enhance sensitivity and the sample is analyzed by spectrophotometer on a Lachat autoanalyzer.

Interferences

1. Salinity has an effect on this analysis.
2. Calcium and magnesium interfere and are removed with EDTA during the analysis.
3. Chlorine is an interference and can be removed with sodium thiosulfate.
4. Turbid samples must be filtered or centrifuged before analysis.
5. Interferences may also be removed with a distillation step before analysis.

Materials

Dry Valleys Lab

Graduated cylinder
125 ml narrow mouth HDPE bottles (acid washed w/ 1% HCL: Fill half-way with 1% HCL, cap, let
sit right-side up for ~1 hour, flip upside-down for ~1 hour, DIW rinse 5 times,. 1% HCL can
be re-used for other acid rinsing since the bottles are new.)
Bell jar filter apparatus
25 mm polysulfone filter funnels
25 mm GF/F filters (combusted at 475° C for 4 h)

MCM Crary Lab

20 ml polycarbonate tubes with snap caps
Assorted volumetric flasks (100, 250, 500 ml).
Lachat QuikChem AE Analyzer.
P1000 Gilson Pipetman and tips
P200 Gilson Pipetman and tips
P5000 Gilson Pipetman and tips
Stir plate

Glassware preparation

1. Wash glassware using standard laboratory technique: Liquinox soap, rinse 3x with tap water; 1 % HCl, then 6x with *DI* water.

Reagents

EDTA Buffer: Dissolve 50.0 g of disodium ethylenediamine tetraacetate (EDTA) and 5.5 g of sodium hydroxide (NaOH) in 900 ml of *DI* water. Dilute to 1000 ml with *DI* water.

Sodium hypochlorite: Dilute 250 g of Clorox bleach to 500 ml with *DI* water. Prepare weekly.

Sodium phenolate: Add 41.5 g of crystalline phenol (C_6H_5OH) to 300 ml *DI* water, in a 500 ml beaker. Add a stir bar and dissolve by stirring at a moderate speed. Turn the stir speed down to slow and, carefully, add 16 g of sodium hydroxide (NaOH). Cool the solution and dilute to 500 ml with *DI* water.

Stock Ammonia solution: NH_4^+ (1000 mg l^{-1}). Dissolve 3.819 g of anhydrous NH_4Cl in 1000 ml of *DI* water.

Standard Ammonia solution: NH_4^+ (10.0 mg l^{-1}). Dilute 10.0 ml of stock ammonia solution to 1.0 l with *DI* water.

Spiking Ammonia solution: NH_4^+ (50 mg l^{-1}). Dilute 5.0 ml of stock ammonia solution to 100 ml with *DI* water.

Procedure

Below steps to be conducted in Dry Valleys Lab

Sample Collection

1. The filtrate produced from the chlorophyll-a filtration (Section 3.1) is collected for nutrient analyses.

2. Gently invert the 1000 ml amber HDPE bottle, thoroughly mixing the sample, and decant 100 ml into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the filter base, which is mounted on a vacuum bell jar, and replace tower. Place an acid washed 125 ml narrow mouth HDPE bottle directly under the filter base. The filter is used as a replicate for Chlorophyll-*a* analysis (see Chlorophyll-*a* method); therefore, filtering must be performed in the dark.

4. Filter the sample under low pressure (<7 in Hg). Collect 100 ml of the filtrate in the narrow mouth HDPE bottle. Do not rinse the filter tower with DIW if you still need to filter the DOC sample. Only rinse the filter towers between sample depths. Carefully remove the bell jar, cap the nutrient bottle (one bottle is used for analysis of all nutrients) and immediately freeze sample (nutrient samples can be kept on the floor of the lab during the filtration process, then placed at -20^{o}C). Store frozen until analysis.

Below steps to be conducted in MCM Crary Lab

Instrument preparation

1. Inspect the pump tubing and PTFE tubing and replace as needed. Pump tubing is inspected by rolling the tube between your thumb and index finger and checking for flat spots. The PTFE tubing is generally changed out completely at the beginning of each season.

2. Fill the diluter bottle and the water reservoir with fresh *DI* water directly from the purifier. Ensure that the waste container has enough empty space for the full day's analysis and that the waste lines are properly positioned in the container. Allow the instrument to warm up for 15 minutes.

3. Turn the instrument on and wait for the instrument computer to boot up. Type 'Q' and then 'tester' and hit enter. The instrument will check all functions for proper operation. Prime the diluter by hitting 'P' and enter. Type 'QQ' to quit tester. Type CC to start analysis program.

4. On the auxiliary (Win95) computer, double click on the Lachat icon. From 'methods' go to 'analysis select' then 'download'. Select the method and wait for method to load.

5. Identify the sample line and the carrier line, rinse the ends well with *DI* water and place into the water reservoir. Cover the top of the reservoir with parafilm to prevent contamination into the reservoir.

6. Identify the NH_4^+ channel reagent lines (3) and place them into a beaker filled with *DI* water.

7. Identify the reagent, carrier and sample line pump cartridges (5). Adjust the line tension all the way loose and lock them in place on the pump. Increase the line tension two clicks.

8. Turn the pump on and run *DI* water through all the lines to clear any air bubbles.

9. Remove the reagent lines from the beaker. Allow a few cm of air to enter the lines, tap ends on side of beaker to remove water from the fittings and place in their respective reagent bottles

10. Allow the air bubbles from step 9 to be expelled from the system.

11. Turn the pump flow to low and allow the system to equilibrate while preparing standards.

Instrument calibration

1. Prepare five NH_4^+-N standards (A-E). To labeled, 100 ml, volumetric flasks, add 2.0, 1.0, 0.50, 0.50 and 0 ml of NH_4^+-N standard ammonia solution, diluting to volume, to make 200, 100, 50, 5 and 0 μg/l standards, respectively.

2. Pour each standard into a separate, labeled, 20 ml polypropylene scintillation vial and place in the labeled spaces in the calibration rack of the instrument.

3. On the auxiliary computer, select calibration, start calibration to begin instrument calibration.

4. Observe the first standard's (A) deflection and ensure that the sample window encloses the high part of the deflection. If window is out of this range, turn the column bypass valve to bypass flow and consult the Lachat operation manual to move window. Allow instrument to equilibrate and repeat steps 3 and 4 until satisfactory measurements are made.

5. After calibration standards are all analyzed, go to the Results/Approval area on the auxiliary computer and ensure that both curves have a correlation coefficient of 0.995 or better to validate calibration.

Sample Analysis

1. Perform dilutions on samples listed on the "Dilutions for Nutrients on Lachat Autoanalyzer" chart located in the operating manual and on page 94 of this manual.

2. Pour each sample into a polycarbonate tube and place in the autosampler tray. The pH of samples, wash water, and calibration standards should be approximately the same.

3. Enter the sample numbers and dilutions under the tray definition section of the auxiliary computer and submit to start analysis.

Quality Control

1. Analyze one reagent blank (*DI* water) per 20 samples. Ensure that the blank measures less than the instrument detection limit to check for instrument drift.

2. Analyze one mid-range standard as continuing calibration verification (CCV) per 20 samples. Ensure a percent recovery between 90 - 110% to continue analysis.

3. Analyze one duplicate analysis per 20 samples. Ensure a relative percent deviation of between 80-120% to confirm precision.

4. Prepare one sample spike for every 20 samples. Fill a 10 ml volumetric flask to mark with sample. Add 40 µl of 50 mg l^{-1} spiking solution and vortex. This makes a 200 µg l^{-1} NH_4^+-N spike. Ensure a percent recovery of between 80-120% to verify no matrix interference.

Instrument Shutdown

1. Remove the reagent lines from the reagent, tapping the ends on the side of the bottle to remove excess reagent and place in a beaker containing *DI* water. Flush the system to remove all reagents from the channel tubing.

2. Turn the pump off, release tension on the pump cartridges and release the cartridges from the pump.

3. Remove reagent lines from the *DI* water and place ends in a clean beaker. Remove the sample and carrier lines from the water reservoir and place ends in a separate beaker. Cover the beakers with parafilm to prevent contamination.

4. Turn off the instrument.

5. Place lids on all reagent and water bottles. Check waste bottle and empty if necessary.

References

Lachat QuikChem AE Automated Ion Analyzer, *Training Manual*.
Lachat QuikChem AE Automated Ion Analyzer, *Methods Manual*.
Lachat QuikChem AE Automated Ion Analyzer, *Software Reference Manual*.
Standard Methods for the Examination of Water and Wastewater," 18th Edition, 1992, 4500-NH_3.

Nitrite and Nitrate Analysis by Autoanalyzer

General Discussion

This method is utilized for the analysis of water samples for nitrate (NO_3^-) and nitrite (NO_2^-) by autoanalyzer. Nitrite levels are determined by diazotization, with sulfanilamide, and then coupled with a diamine to produce a pink dye analyzed by spectrophotometer on a Lachat autoanalyzer. Nitrate levels are determined by reducing nitrate to nitrite by passing the sample through a copperized cadmium column and analyzing for nitrite, as above, giving a nitrate + nitrite (N+N) concentration. The nitrate concentration is determined by subtracting the nitrite concentration from the N+N concentration.

Interferences

The efficiency of the cadmium column can be affected when samples contain sulfur, residual chlorine, heavy metals, or oil and grease. Samples can be treated by adding EDTA to the sample for metals removal, sodium thiosulfate for chlorine removal, organic extraction for oil and grease removal, and dilution for sulfur removal. The column efficiency must be verified.

Materials

Dry Valleys Lab

Graduated cylinder
125 ml narrow mouth HDPE bottles (acid washed w/ 1% HCL: Fill half-way with 1% HCL, cap, let sit right-side up for ~1 hour, flip upside-down for ~1 hour, DIW rinse 5 times,. 1% HCL can be re-used for other acid rinsing since the bottles are new.)
Bell jar filter apparatus
25 mm Polysulfone filter towers
25 mm GF/F filters (combusted at 475° C for 4 h)

MCM Crary Lab

20 ml polycarbonate tubes with snap caps
Assorted volumetric flasks (100, 250, 1000 ml)
Glass test tubes: 13 x 100 mm
Lachat QuikChem AE Analyzer.
P1000 Gilson Pipetman and tips
P200 Gilson Pipetman and tips
P5000 Gilson Pipetman and tips
Stir plate

Glassware preparation

Wash glassware using standard laboratory technique: Liquinox soap, rinse 3× with tap water then 3× with *DI* water.

Reagents

Imidazole Buffer: Add 13.6 g of imidazole to 1000 ml beaker containing 800 ml of deionized *DI* water. Add a stir bar to the beaker and stir at medium speed to mix. Adjust the pH of the solution to 8.0 with 1.0 N HCl and adjust the volume to 1000 ml with *DI* water.

Sulfanilamide color reagent: Add 100 ml of 85% phosphoric acid to 600 ml *DI* water in a 1 beaker and vortex. Add 40.0 g sulfanilamide and 1.0 g N-(1-naphthyl)-ethylenediamine dihydrochloride (NED). Place a stir bar in the beaker and stir at medium speed until dissolved. Dilute to 1000 ml with *DI* water in a volumetric flask. Store in a dark bottle. Reagent should be discarded when pink.

Stock Nitrite solution: NO_2^--N (1000 mg l^{-1}). Dissolve 6.072 g KNO_2 (dried in a desicator for 24 h) in nitrite-free water and dilute to 1000 ml.

Stock Nitrate solution: NO_3^--N (1000 mg l^{-1}). Dissolve 7.218 g of KNO_3 (dried in an oven at 105° C for 24 h) in water and dilute to 1000 ml.

Standard Nitrite-Nitrate solutions: (10 mg l^{-1}). Dilute 10.0 ml of each stock solution to 1000 ml with *DI* water.

Spiking Nitrite-Nitrate solutions: (50 mg l^{-1}). Dilute 5.0 ml of each stock solution to 1000 ml with *DI* water.

Procedure

Below steps to be conducted in Dry Valleys Lab

Sample Collection

1. The filtrate produced from the chlorophyll-a filtration (Section 3.1) is collected for Nutrient analyses.

2. Gently invert the 1000 ml amber HDPE bottle, thoroughly mixing the sample, and decant 100 ml into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the filter base, which is mounted on a vacuum bell jar, and replace tower. Place an acid washed 125 ml narrow mouth HDPE bottle directly under the filter base. The filter is used as a replicate for Chlorophyll-*a* analysis (see Chlorophyll-*a* method); therefore, filtering must be performed in the dark.

4. Filter the sample under low pressure (<7 in Hg). Collect 100 ml of the filtrate in the narrow mouth HDPE bottle. Do not rinse the filter tower with DIW if you still need to filter the DOC sample. Only rinse the filter towers between sample depths. Carefully remove the bell jar, cap the nutrient bottle (one bottle is used for analysis of all nutrients) and immediately freeze sample (nutrient samples can be kept on the floor of the lab during the filtration process, then placed at -20^{o}C). Store frozen until analysis.

Below steps to be conducted in MCM Crary Lab

Instrument Preparation

1. Inspect the pump tubing and PTFE tubing and replace as needed. Pump tubing is inspected by rolling the tube between your thumb and index finger and checking for flat spots. The PTFE tubing is generally changed out completely at the beginning of each season.

2. Fill the diluter bottle and the water reservoir with fresh *DI* water directly from the purifier. Ensure that the waste container has enough empty space for the full days analysis and that the waste lines are properly positioned in the container. Allow the instrument to warm up for 15 minutes.

3. Turn the instrument on and wait for the instrument computer to boot up. Type 'Q' and then 'tester' and hit enter. The instrument will check all functions for proper operation. Prime the diluter by hitting 'P' and enter. Type 'QQ' to quit tester. Type CC to start analysis program.

4. On the auxiliary (Win95) computer, double click on the Lachat icon. From 'methods' go to 'analysis select' then 'download'. Select the method and wait for method to load.

5. Identify the sample line and the carrier line, rinse the ends well with *DI* water and place into the water reservoir. Cover the top of the reservoir with parafilm to prevent contamination into the reservoir.

6. Identify the NO_2^-/NO_3^- channel reagent lines (2 for each channel) and place them into a beaker filled with *DI* water.

7. Identify the reagent, carrier and sample line pump cartridges (7). Adjust the line tension all the way loose and lock them in place on the pump. Increase the line tension two clicks.

8. Turn the pump on and run *DI* water through all the lines to clear any air bubbles. **Note**: Ensure that the cadmium bypass valve is in bypass position so that no water or air passes through the column if present.

9. Remove the reagent lines from the beaker. Allow a few cm of air to enter the lines, tap ends on side of beaker to remove water from the fittings and place in their respective reagent bottles (sulfanilamide and imidazole (2 lines in each)).

10. Allow the air bubbles from step 9 to be expelled from the system.

11. If necessary, install a cadmium column. Remove the old column, if present. Using a beaker to catch waste, turn the bypass valve in-line, allowing reagent to flow out the line. Being careful not to introduce air into the column, remove the column spacer line and screw one end of the column onto the flowing reagent line. Make sure that there is flow out of the other end of the column and connect to the outflow line.

12. Turn the pump flow to low and allow the system to equilibrate while preparing standards.

Instrument calibration

1. Prepare five NO_2^--N standards (A-E). To labeled, 100 ml, volumetric flasks, add 2.0, 1.0, 0.50, 0.50 and 0 ml of NO_2^--N standard nitrite solution, diluting to volume, to make 200, 100, 50, 5 and 0 $\mu g\ l^{-1}$ standards, respectively.

2. Prepare five NO_3^--N standards (F-J). To labeled, 100 ml, volumetric flasks, add 8.0, 4.0, 2.0, 1.0 and 0 ml of NO_3^--N standard nitrate solution, diluting to volume, to make 800, 400, 200, 100 and 0 $\mu g\ l^{-1}$ standards, respectively.

2. Pour each standard into a separate, labeled, 20 ml polypropylene scintillation vial and place in the labeled spaces in the calibration rack of the instrument.

3. On the auxiliary computer, select calibration, start calibration to begin instrument calibration.

4. Observe the first standard's (A) deflection and ensure that the sample window encloses the high part of the deflection. If window is out of this range, turn the column bypass valve to bypass flow and consult the Lachat operation manual to move window. Allow instrument to equilibrate and repeat steps 3 and 4 until satisfactory measurements are made.

5. After calibration standards are all analyzed, go to the Results/Approval area on the auxiliary computer and ensure that both curves have a correlation coefficient of 0.995 or better to validate calibration.

Cadmium Column Efficiency

1. Fill a 13 ×100 mm test tube with the 100 $\mu g\ l^{-1}$ NO_2^--N standard and place in space 1 of the sample rack on the autosampler. Repeat for the 100 $\mu g\ l^{-1}$ NO_3^--N standard and a blank (*DI* water), placing them in spaces 2 and 3, respectively.

2. On the auxiliary computer, go to the tray definition section and enter the sample names for space 1,2 and 3. Submit the tray to start the analysis.

3. Calculate the column efficiency (CE) by the following equation:

$$CE = \frac{\left[\left(NO_3^- + NO_2^-\right) - NO_2^-\right]}{\left[NO_2^-\right]} \times 100$$

The acceptable range for CE is 90 - 110 to validate nitrite values. If the column efficiency is outside of this range, a new column must be installed, a new calibration performed and the CE procedure repeated.

Sample Analysis

1. Perform dilutions on samples listed on the "Dilutions for Nutrients on Lachat Autoanalyzer" chart located in the operating manual and on page 94 of this manual.

2. Pour each sample into a 13 x 100 mm test tube and place in the autosampler tray.

3. Enter the sample numbers and dilutions under the tray definition section of the auxiliary computer and submit to start analysis.

Quality Control

1. Analyze one reagent blank (*DI* water) per 20 samples. Ensure that the blank measures less than the instrument detection limit to check for instrument drift.

2. Analyze one mid-range standard, per analyte, as continuing calibration verification (CCV) per 20 samples. Ensure a percent recovery between 90 - 110% to continue analysis.

3. Analyze one duplicate analysis per 20 samples. Ensure a relative percent deviation of between 80-120% to confirm precision.

4. Prepare one sample spike for every 20 samples. Fill a 10 ml volumetric flask to mark with sample. Add 40 µl of 50 mg l^{-1} spiking solution and vortex. This makes a 200 µg l^{-1} NH_4^+-N spike. Ensure a percent recovery of between 80-120% to verify no matrix interference.

Instrument Shutdown

1. Turn the column bypass valve to the bypass position.

2. Remove the reagent lines from the reagent, tapping the ends on the side of the bottle to remove excess reagent and place in a beaker containing *DI* water. Flush the system to remove all reagents from the channel tubing.

3. Turn the pump off, release tension on the pump cartridges and release the cartridges from the pump.

4. Remove reagent lines from the *DI* water and place ends in a clean beaker. Remove the sample and carrier lines from the water reservoir and place ends in a separate beaker. Cover the beakers with parafilm to prevent contamination.

5. Turn off the instrument.

6. Place lids on all reagent and water bottles. Check waste bottle and empty if necessary.

References

Lachat QuikChem AE Automated Ion Analyzer, *Training Manual*.
Lachat QuikChem AE Automated Ion Analyzer, *Methods Manual*.
Lachat QuikChem AE Automated Ion Analyzer, *Software Reference Manual*.
"Standard Methods for the Examination of Water and Wastewater," 18th Edition, 1992, 4500-NO_2^- B. and 4500-NO_3^- E.

Soluble Reactive Phosphorus by Mixed Molybdate: Manual Method

General Discussion

Materials
25 ml test tubes (acid soaked and rinsed)
500 ml amber glass bottles
Assorted beakers (100, 250, 500, 1000 ml)
Assorted graduated cylinders (100, 500, 1000 ml)
Assorted volumetric flasks(100, 250, 1000 ml)
P10 ml Gilson Pipetman and tips
Spectrophotometer with 10 cm cells

Reagents
5N Sulfuric acid (H_2SO_4): Carefully add 140 ml concentrated H_2SO_4 to 800 ml DIW in a 1000 ml graduated cylinder. Bring to 1000 ml with *DI* water. After cool, store in a polyethylene bottle on the shelf.

Ammonium molybdate solution: Bring 15 g ammonium molybdate to 500 ml in a 500 ml graduated cylinder and vortex. Transfer to a clean 500 ml glass bottle and store refrigerated.

Potassium antimonyl tartrate solution: Bring 2.73 g of Potassium antimonyl tartrate to 500 ml in a 500 ml graduated cylinder and vortex. Transfer to a clean 500 glass bottle and store at 4° C.

Stock solution (0.1 mM): Bring 0.0136 g K_2PO_4 to 1000 ml with *DI* water.

Mixed Reagent: In a 100 ml graduated cylinder, carefully vortex 50 ml of 5 N H_2SO_4, 20 ml of ammonium molybdate solution, and 10 ml of potassium antimonyl tartrate solution. Dissolve 1.08 g of Ascorbic acid into this solution and bring the solution to 100 ml with *DI* water. This solution should be yellowish not blue. If it is blue, there has been some phosphate contamination. **Make this fresh at the time of each assay.**

Procedure
Below steps to be conducted in Dry Valleys Lab

Sample Collection

1. The filtrate produced from the chlorophyll-a filtration (Section 3.1) is collected for Nutrient analyses.

2. Gently invert the 1000 ml amber HDPE bottle, thoroughly mixing the sample, and decant 100 ml into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the filter base, which is mounted on a vacuum bell jar, and replace tower. Place an acid washed 125 ml narrow mouth HDPE bottle directly under the filter base. The filter is used as a replicate for Chlorophyll-*a* analysis (see Chlorophyll-*a* method); therefore, filtering must be performed in the dark.

4. Filter the sample under low pressure (<7 in Hg). Collect 100 ml of the filtrate in the narrow mouth HDPE bottle. Do not rinse the filter tower with DIW if you still need to filter the DOC sample. Only rinse the filter towers between sample depths. Carefully remove the bell jar, cap the nutrient bottle (one bottle is used for analysis of all nutrients) and immediately freeze sample (nutrient samples can be kept on the floor of the lab during the filtration process, then placed at -20^{o}C). Store frozen until analysis.

Below steps to be conducted in MCM Crary Lab

1. Add the following volumes of stock solution (Table 16) to 10 ml of *DI* water in acid rinsed 25 ml test tubes. These tubes will serve as calibration standards.

2. Pipet exactly 10 ml of sample into 25 ml test tubes.

3. Add 1 ml of mixed reagent to each tube and vortex.

4. Allow color to develop for at least 20 minutes but not more than 3 h.

Table 16. Volume of stock solution used to prepare standard solutions.

Tube #	Vol. of 0.1 mM stock (ml)	Final Concentration. (μM)
1	0	0
2	0	0
3	0.01	0.1
4	0.01	0.1
5	0.02	0.25
6	0.02	0.25
7	0.05	0.5
8	0.05	0.5
9	0.1	1.0
10	0.1	1.0

5. Calibrate spectrophotometer to read zero absorbance (100% transmittance) against *DI* water. Measure the absorbance of the calibration standards and samples at 885 nm. Use the same cuvette for all readings. Rinse cuvette with *DI* water between samples.

6. Use the standard curve regression equation to calculate PO_4^{-3} values (μM) for each sample.

Dilutions for Nutrients on Lachat Autoanalyzer

ELB:
NH4: 3-14m=no dilution; 15m and below, dilute 1 in 10 (sample:DIW)
NO2: 3-14m=no dilution; 15m and below, dilute 1 in 10 (sample:DIW)
NO3: 3-14m=no dilution; 15m and below, dilute 1 in 10 (sample:DIW)
SRP: NO DILUTIONS

WLB:
NH4: 3-10m=no dilution; 12m and below, dilute 1 in 10 (sample:DIW)
NO2: NO DILUTIONS
NO3: 3-10m=no dilution; 12m and below, dilute 1 in 10 (sample:DIW)
SRP: NO DILUTIONS

FRX:
NH4: 0-9m no dilution; 10-**11m**, dilute 1 in 10 (sample:DIW); **11m** and below, dilute 1:100 *(?)*
NO2: NO DILUTIONS
NO3: NO DILUTIONS
SRP: 0-9m no dilution; 10m and below, dilute 1 in 10 (sample:DIW)

HOR:
NH4: NO DILUTIONS
NO2: NO DILUTIONS
NO3: NO DILUTIONS
SRP: NO DILUTIONS

Vanda:
NH4: 3-45m=no dilution; 46-60m, dilute 1 in 10 (sample:DIW) 61m and below dilute 1:100
NO2: NO DILUTIONS
NO3: 3-**55m**=no dilution; **55m** and below, dilute 1 in 10 (sample:DIW) *(55m - salinity still low and doesn't need dilution because of curve)*
SRP: 3-45m=no dilutions; 46m and below, dilute 1 in 10

Dissolved Oxygen by Mini-Winkler Titration

General Discussion

Materials

Sampling Hut

Benchcoat
Kimwipes
P1000 Gilson Pipetman and tips (2-one for each reagent)
Vinyl gloves (at least 3 pair)
Glass scintillation vials (20 ml vials with HDPE cone caps)
Serum/Scintillation vial carrier
Plastic scintillation tray
Ziplock bag for waste

Dry Valleys lab

Benchcoat
Kimwipes
P1000 Gilson Pipetman and tips (3-one for each reagent)
DI squirt bottle
Eyedropper
Forceps
Gilson micro-buret (2 ml)
Lamp or good sunlight
Latex/vinyl gloves (at least 3 pairs)
Magnetic stir plate
Micro stir bar
Mini beakers (10 ml)
Waste bottle
Ziplock for lab waste

Reagents

Sampling Hut

Alkaline-iodide-azide solution: In a volumetric flask bring the following reagents to 250 ml using *DI* water (KEEP IN DARK AS POTASSIUM IODIDE IS LIGHT SENSITIVE!!):

- 2.5 g sodium azide (NaN_3)
- 25 g potassium iodide (KI)
- 80 g sodium hydroxide (NaOH)

Manganous solution (Mn^{++}): In a volumetric flask bring 100 g of $MnCl_2 \cdot 4H_2O$ to 250 ml using *DI* water.

Dry Valleys Lab

Alkaline-iodide-azide solution (see above)
Manganous solution (Mn^{++}) (see above)

Phosphoric acid (H_2PO_4): Concentrated
Sodium thiosulfate titrant ($Na_2S_2O_3 \cdot 5H_2O$): In 0506 we started using a pre-maid sodium thiosulfate (1.0 N) solution and diluting it to .01 N.
Starch solution (1%): Dissolve 1 g of laboratory–grade soluble starch in 100 ml of hot *DI* water.

Procedure
Sample Collection

1. Place the Niskin bottle tubing from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times) to the bottom of the 20 ml scintillation vial. Allow the water overflow, displacing the initial sample volume 2x (about 5 seconds). Carefully remove the tubing, keeping turbulence to a minimum, making sure the vial is completely filled with no air bubbles.

2. Fix the sample by adding the following reagents (directly into sample):
   0.9ml of the Mn^{2+} solution
   0.9ml of the alkali-iodide-azide solution

3. Cap the sample and invert several times to ensure mixing. Use benchcoat on the table top and kimwipes to mop up the excess fluid that is displaced.

4. Place sample into the Serum/Scintillation vial carrier for transport.

Below steps to be conducted in Dry Valleys Lab
Sample Analysis

1. Prepare each sample for titration by adding 1 ml of phosphoric acid. Invert each sample several times and wait for precipitate to dissolve.

2. Dilute the concentrated sodium thiosulfate solution by bringing 1 ml of the concentrated solution to 100 ml in a volumetric flask using *DI* water to achieve a .01N solution.

3. Sample titration: Place a kimwipe on the stir plate and position the plate such that it is in direct light (a desk lamp may be used) to easily observe colorimetric change.

5. Withdraw 2 ml of the diluted sodium thiosulfate into the microburet. **Note:** To minimize contamination of the thiosulfate, fill a scintillation vial with thiosulfate and use instead of the diluted solution stock.

6. Pipet 1 ml of sample into a small glass beaker or scintillation vial, and place a micro stir bar into the sample.

7. While keeping the tip of the microburet in the sample, slowly titrate to a pale straw yellow color; add 1-2 drops of starch solution (heat starch solution prior to addition to make sure starch is in solution), this will turn the sample dark blue; then titrate to endpoint (first disappearance of blue color). Place a mini beaker containing 2 ml of *DI* water along side the

sample beaker. This will provide a reference color during sample titration. **Note**: If the sample does not turn dark blue, the starch may have precipitated. Resuspend the starch by heating solution (hot, but not boiling).

8. Record the volume of titrant, and repeat titration. Titrate each sample at least 2 times. Variation among the replicates should be less than ± 0.5%.

Note: After each titration, rinse the buret with *DI* water (into a waste bottle), and wipe dry with a kimwipe. Then refill with the thiosulfate solution, and wipe the buret dry again. Also, pour the titrated sample into the waste bottle; rinse the beaker with *DI* water and wipe dry.

9. Determine oxygen concentration as follows.

$$\text{Dissolved Oxygen}\left(\text{mgO}_2\ \text{l}^{-1}\right) = \frac{(\text{ml titrant})(M_{thio})(8000)}{(\text{ml sample titrated})\left(\frac{\text{ml of bottle} - 1.8}{\text{ml of bottle}}\right)}$$

where M_{thio} is the molarity of thiosulfate, and 8000 converts thiosulfate equivalents to oxygen equivalents and ml filtered to liters.

References

Carpenter, J.H. 1965. The Chesapeake Bay Institute technique for the Winkler Dissolved Oxygen method. Limnology and Oceanography. 10(1):141-143.

Hydrogen Ion concentration (pH)

General Discussion

The following method utilizes a pH meter consisting of a potentiometer, a polymer body Ag/AgCl probe and a temperature compensating probe.

Materials

Sampling hut

Glass scintillation vials (20 ml vials with HDPE cone caps)
Serum/Scintillation vial carrier
Scintillation tray

Dry Valleys Lab

DI water
Kimwipes
Portable pH meter with appropriate pH electrode, ATC probe and cables (Beckman PSI 10)
Waste Beaker

Reagents

Dry Valleys Lab

pH Buffers (4, 7, 10 pH)
Probe storage and filling solution

Procedure

Sample Collection

1. Place the Niskin bottle tubing from a well-mixed Niskin bottle (i.e., drain ~ 10 ml first and invert Niskin ~ 5 times) to the bottom of the 20 ml scintillation vial. Allow the water to overflow, displacing the initial sample volume 2x (about 5 seconds). Carefully remove the tubing, keeping turbulence to a minimum, making sure the vial is completely filled. Place sample into the Serum/Scintillation vial carrier for transport.

Below steps to be conducted in Dry Valleys Lab

2. Calibrate (double endpoint) the pH meter and probe using pH 4 and 7 buffers.

3. Place the pH probe into the sample and manually agitate in an up and down motion (~1 cm). The pH and temperature probes will not simultaneously fit into a scintillation vial. Therefore, place the temperature probe into the next sample to obtain a temperature reading.

4. Once the pH and temperature probe have stabilized, record the values.

5. Rinse the electrode with *DI* water between standards and samples and blot dry with Kimwipes.

Underwater Time Series PAR Logged During Primary Production Experiment

General Discussion

Materials
Licor LI-1400 Data logger with 30 m cable
Licor LI-193SA spherical quantum sensor
Licor LI-190SA quantum sensor
Leveling fixture
Lowering Frame
Tarpaulin (opaque)

Procedure
- **Clear the memory** of the LI-1400 by pressing FCT and scrolling with the right arrow until you see CLEAR MEMORY. Press Enter. The cursor should be on "Clear=." Use the right arrow to choose "All." Press the down arrow three times to move the cursor to "!Clear Mem ?NO !" and use the right arrow to change the NO to YES. Press enter. The instrument should say "Clearing Memory..."
- Set the **date and time** (this can also be done through the software) by pressing SETUP and scrolling with the right arrow until you see CLOCK. Press enter. The cursor should be on "Date." Press enter. On the screen that says "Editing Date," enter the year, month and day. Press enter. Use the down arrow to scroll to "time." Press enter. On the screen that says "Editing Time," enter the time. Press enter. Press ESC to get back to the SETUP menu.
- Note that the LI-1400 screen will blink on and off when the **batteries** are low.

1. Configure the LI-1400 to record light data on channels 1 (underwater sensor) and 2 (ambient sensor) and to log data. This can be done using the logger key pad:

- SETUP → CHANNELS (Press enter to get to the "Setup Channels" screen and use the up and down arrows to select I1):
    - I1 = Light: Use the right and left arrows to select "light," then press enter to get to the "Set up Light" screen. Press enter to edit Descr, then press enter to return to the "Set up Light" screen and ↓ to get to next parameter. Follow the same method for Mult and Label. Log Routine, Calc and Min/Max can be changed by using the right and left arrows to toggle between options:
        - Desc= UW
        - Mult= enter multiplier of UW sensor
        - Label= UM (umol photons/m^2/s)
        - Average= 1 sec (only used when viewing data in instantaneous mode)
        - Log Routine= LR1 (the start and stop times, sampling and logging period will be set in the LOGGING setup described below).
        - Calc= Mean (Defines form in which data are collected and stored).
        - Min/Max= No (Stores min and max data points during logging period).
        - Tcoef= Not used if mean values are stored to memory.

        Press esc to return to "Set up Channels"

    - I2 = Light: Use the right and left arrows to select "light," then press enter to get to the "Set up Light" screen. Press enter to edit Descr, then press enter to return to the "Set up Light" screen and ↓ to get to next parameter. Follow the same method for Mult and Label. Log Routine, Calc and Min/Max can be changed by using the right and left arrows to toggle between options:
        - Desc= Ambient
        - Mult= enter multiplier of UW sensor
        - Label= UM (umol photons/m^2/s)
        - Average= 1 sec (only used when viewing data in instantaneous mode)
        - Log Routine= LR1 (the start and stop times, sampling and logging period will be set in the LOGGING setup described below).
        - Calc= Mean (Defines form in which data are collected and stored).
        - Min/Max= No (Stores min and max data points during logging period).
        - Tcoef= Not used if mean values are stored to memory.

        Press esc to return to "Set up Channels"
        Press esc to return to Setup Menu

2. Configure the LI-1000 to Log Mean PAR values every 10 minutes. This can be done using the logger key pad:

- SETUP → LOGGING (press enter):
    - "Set up Logging" (use the left and right arrows to set the following parameters for Logging, Auto Print, Overwrite and PwrDelay. Use the down arrow to get to the next parameter).

        - Logging= ON
        - Auto Print= NO
        - Overwrite= YES
        - PwrDelay= 0 sec
        - LR1: press enter and set up a Log Routine as follows:
    - "Set Log Routn" (Press enter to edit Start Time and Stop Time then press enter to return to the "Set up Light" screen and ↓ to get to next parameter. Sampling Period and Logging Period can be changed by using the right and left arrows to toggle between options.)
        - Start Time= make Start Time = Stop Time for logging to occur 24 hours a day (use the back arrow to erase the current time, and then enter the desired time).
        - Stop Time= make Start Time = Stop Time for logging to occur 24 hours a day (use the back arrow to erase the current time, and then enter the desired time).
        - Sampling Period= 5 sec
        - Logging Period= 5 min

    Press esc to return to "Set up Logging."

- Press esc to return to Setup Menu.
- **Press VIEW: You will see the message "Implementing New Setup"** if you changed any of the configurations.

3. The time series logged data is recorded inside the weatherport sampling hole. Once all of the water samples have been collected, lower the underwater sensor frame to a depth of 10 m (7 m at Lake Fryxell) below the piezometric water level and secure to the winch frame. Place the ambient sensor frame as far away from the weatherport as possible and attach both cables to the appropriate channels on the LI-1000. Make sure you give yourself enough time, once the **reset time** is programmed, to position the sensors and data logger.

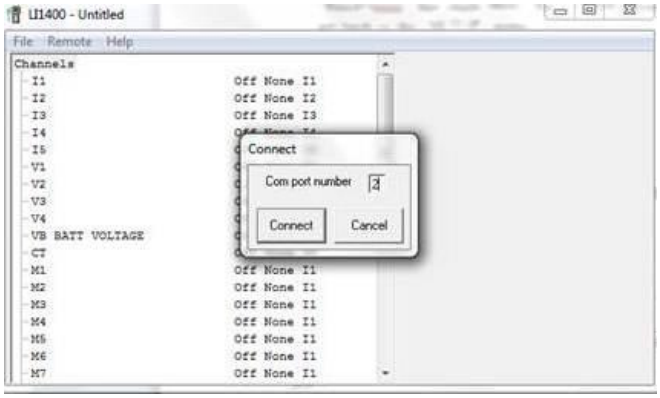
4. Allow the LI-1000 to log for a 24 h period. **Stop logging by going to SETUP → LOGGING and setting the Logging to OFF. Note**: Due to logistic and time constraints it is difficult to immediately start PAR logging once the PPR samples are placed in the incubation hole.

Download the recorded data to a computer using the LI-1400 Data Logger Windows Interface Software program.

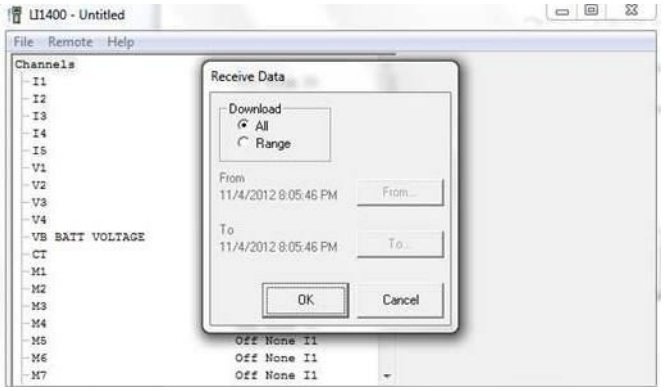
- Change the **baud rate** on the LI-1400 to 9600 by pressing SETUP and scrolling with the right arrow until you see HARDWARE. Press enter. Press the right arrow until the

"Baud=xxxx" line reads 9600. The LI-1400 automatically saves this setting. Press ESC to get back to the "SETUP" menu.

- Connect the LI-1400 to the computer and open the **LI-1400 Data Logger Windows Interface Software program**. Click on REMOTE → CONNECT and enter the correct com port (2). A message that says synchronizing will be displayed until communication is established.



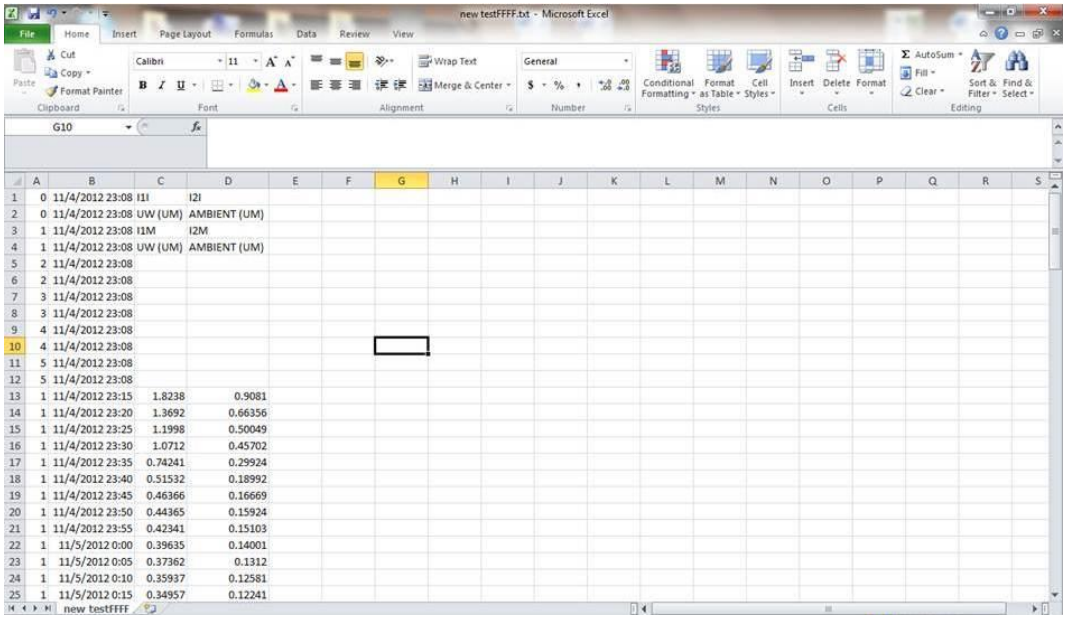
- Click on REMOTE → RECEIVE DATA.



- When prompted, select a directory and choose a file name. Click save. Data is saved as a tab delimited text (.txt) file that can be opened in a spreadsheet. Once the data are successfully downloaded, data can be cleared using the Windows Interface program by clicking on REMOTE → CLEAR DATABASE, or by using the logger key pad as described above.

When the data are opened in a spreadsheet, the 3 character code for each channel should be I1M and I2M. In the code I1M and I2M, the first" I" signifies a current channel, the number signifies the channel used, and the "M" signifies that the mean was logged for the channel. Data are listed for the channels in the columns designated with the 3 character codes. The "Desc" (UW and Ambient) and the "Label" (UM) entered during "setup channels" are listed below the 3 character code.

In the spreadsheet, a "0" in column A shows which channels were active; a "1" in column A shows which channels were logging on LR1, a "2" shows which channels were logging on LR2, and so on through LR5. Data are designated with a "1" in column A because they were logged with LR1.



	A	B	C	D
1	0	11/4/2012 23:08	I1I	I2I
2	0	11/4/2012 23:08	UW (UM)	AMBIENT (UM)
3	1	11/4/2012 23:08	I1M	I2M
4	1	11/4/2012 23:08	UW (UM)	AMBIENT (UM)
5	2	11/4/2012 23:08		
6	2	11/4/2012 23:08		
7	3	11/4/2012 23:08		
8	3	11/4/2012 23:08		
9	4	11/4/2012 23:08		
10	4	11/4/2012 23:08		
11	5	11/4/2012 23:08		
12	5	11/4/2012 23:08		
13	1	11/4/2012 23:15	1.8238	0.9081
14	1	11/4/2012 23:20	1.3692	0.66356
15	1	11/4/2012 23:25	1.1998	0.50049
16	1	11/4/2012 23:30	1.0712	0.45702
17	1	11/4/2012 23:35	0.74241	0.29924
18	1	11/4/2012 23:40	0.51532	0.18992
19	1	11/4/2012 23:45	0.46366	0.16669
20	1	11/4/2012 23:50	0.44365	0.15924
21	1	11/4/2012 23:55	0.42341	0.15103
22	1	11/5/2012 0:00	0.39635	0.14001
23	1	11/5/2012 0:05	0.37362	0.1312
24	1	11/5/2012 0:10	0.35937	0.12581
25	1	11/5/2012 0:15	0.34957	0.12241

Underwater Profile PAR and Attenuation

General Discussion

Materials

Licor LI-1400 Data logger with 50 m cable
Licor LI-193SA spherical quantum sensor
Licor LI-190SA quantum sensor
Leveling fixture
Lowering Frame
Tarpaulin (opaque)

Procedure

3. Configure the LI-1400 to record light data on channels 1 (underwater sensor) and 2 (ambient sensor) and to provide instantaneous point values. This can be done using the logger key pad:

- SETUP → CHANNELS (Press enter to get to the "Setup Channels" screen and use the up and down arrows to select I1):
    - I1 = Light: Use the right and left arrows to select "light," then press enter to get to the "Set up Light" screen. Press enter to edit Descr, then press enter to return to the "Set up Light" screen and ↓ to get to next parameter. Follow the same method for Mult and Label. Average and Log Routine can be changed by using the right and left arrows to toggle between options. The rest of the parameters are not used when doing instantaneous measurements:
        - Desc= UW
        - Mult= **enter multiplier of UW sensor**
        - Label= UM (umol photons/m^2/s)
        - Average= 1 sec (this gives an instantaneous value updated every second)
        - Log Routine= none
        - Calc= (only used if using a log routine)

- Min/Max= (only used if using a log routine)
- Tcoef= (only used if using a log routine)

Press esc to return to "Set up Channels"

- SETUP → CHANNELS (Press enter to get to the "Setup Channels" screen and use the up and down arrows to select I2):
  - I2 = Light: Use the right and left arrows to select "light," then press enter to get to the "Set up Light" screen. Press enter to edit Descr, then press enter to return to the "Set up Light" screen and ↓ to get to next parameter. Follow the same method for Mult and Label. Average and Log Routine can be changed by using the right and left arrows to toggle between options. The rest of the parameters are not used when doing instantaneous measurements:
    - Desc= Ambient
    - Mult= **enter multiplier of UW sensor**
    - Label= UM (umol photons/m^2/s)
    - Average= 1 sec (this gives an instantaneous value updated every second)
    - Log Routine= none
    - Calc= (only used if using a log routine)
    - Min/Max= (only used if using a log routine)
    - Tcoef= (only used if using a log routine)

    Press esc to return to "Set up Channels"

- Press esc to return to Setup Menu.
- **Press VIEW: You will see the message "Implementing New Setup**" if you changed any of the configurations.
- Use the right arrow to toggle to "New Data" and press enter.
- Any of the four lines (use up and down arrows) can be toggled between I1I (channel 1), I2I (channel 2), the current date and time, and how much memory has been used. Both the UW and Ambient sensors can be viewed at once by setting line 1 to I1I and line 2 to I2I. (In the code I1I and I2I, the first" I" signifies a current channel, the number signifies the channel used, and the second "I" signifies that the averaging of the data is set to 1 second).

2. Collect the PAR profile data in a covered incubation hole (use a tarp). Situate the incident surface PAR sensor nearby on a level surface, and lower the wet sensor frame into the water. Collect PAR data on 0.5 m increments beginning at just under the surface of the water in the ice hole and continue until the underwater PAR = 0. Once the underwater sensor stabilizes, record both the underwater and incident PAR values.

Note: Always record the time of day and ambient weather conditions (i.e., cloud cover, sunshine, etc.) when profile is collected.

Biospherical Profiling Natural Fluorescence

General Discussion

The PNF-300 is an integrated optical system specifically designed to measure natural fluorescence - the fluorescence from the phytoplankton community stimulated by available sunlight. Research has shown that natural fluorescence is correlated to ^{14}C photosynthetic rates and chlorophyll concentrations. Unlike strobe fluorometers, a natural fluorometer measures fluorescence emitted under the ambient light conditions which is driving *in situ* photosynthesis.

Materials

Biospherical PNF-300:

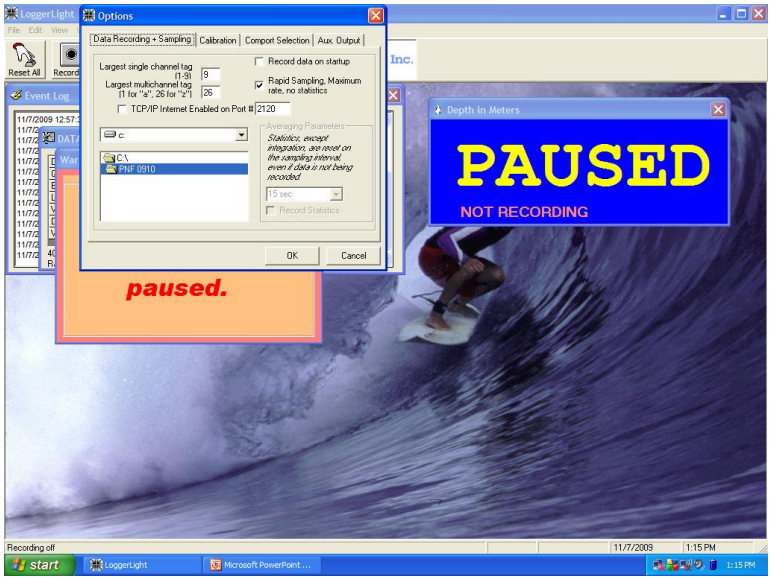
- Underwater PNF Instrument with cable
- Surface PAR sensor with cable
- Deck box
- Computer cable
- Instruction manual
- PC labtop computer
- LoggerLight program

Procedure

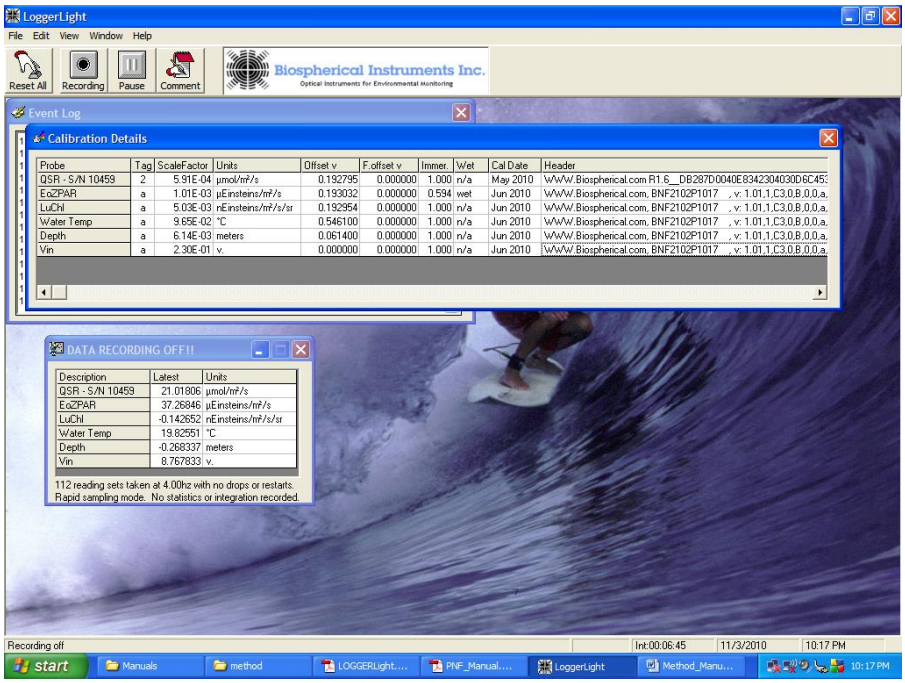
Instrument Calibration

1. Install the PNF-300 software, "LoggerLight," onto the PC laptop.

2. Make a folder for PNF files on the c: drive and go to "Edit" → "Options." In the Data Recording and Sampling tab, choose this folder to save the PNF cast files to. If you have newly installed the LoggerLight program, this option will be given to you the first time you open the program.



3. The PNF 300 should be calibrated every year by Biospherical Instruments Inc. and a calibration sheet should be supplied. The calibration information is stored in the PNF instrument, and can be checked against the calibration sheet by going to "View" → "Calibration." This window displays the calibration information that is stored in the instrument, as well as any values for field offset (F.offset v) (see "Zero and Dark Offset correction" below) which are only stored in the registry of the computer.



NOTE: Check the serial number on the surface sensor unit to ensure it matches the serial number on the calibration certificate. There is more than one surface sensor in Crary Lab, but only one is sent out each year for calibration with the PNF.

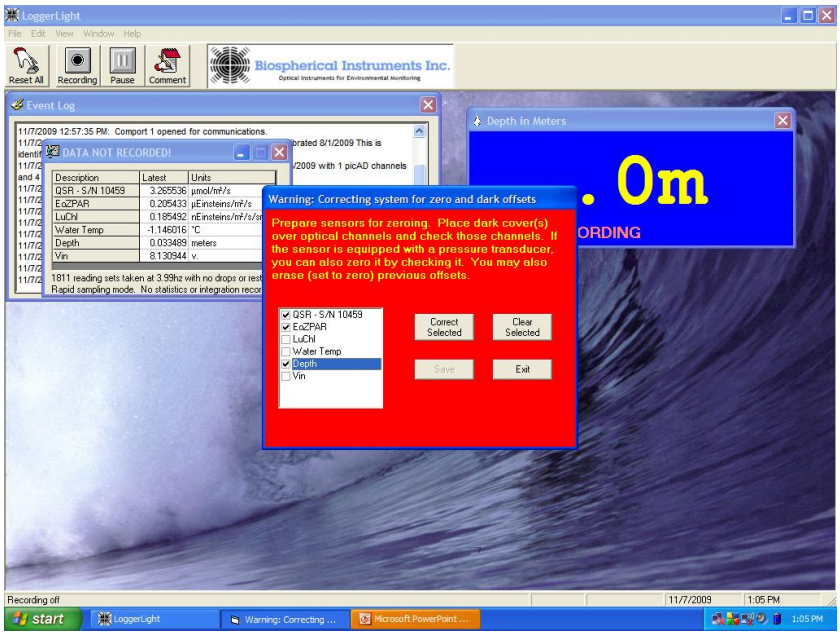
Data Collection

Pre-Deployment

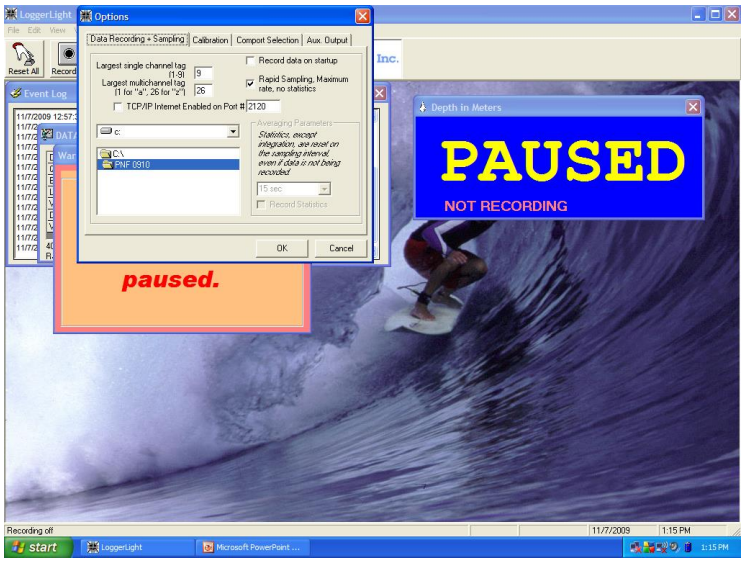
1. Connect the surface PAR sensor and PNF to the deck box, connect the deck box to the computer, and position the cable so the PNF can be easily deployed. Start the LoggerLight program.

2. Perform the Zero and Dark Offset correction:
    a. In LoggerLight, go to "Edit" → "Dark Correction"
    b. Check the UW PAR sensor box (EoZPAR)
    c. Check the surface PAR sensor box (QSR – S/N 10459)
    d. Check the depth sensor box (Depth)
    e. Cover sensors – ensure that both the surface and UW PAR sensors are covered with the supplied black covers, and that the PNF is out of the water.
    f. Press "Correct Selected"
    g. Press "Save"

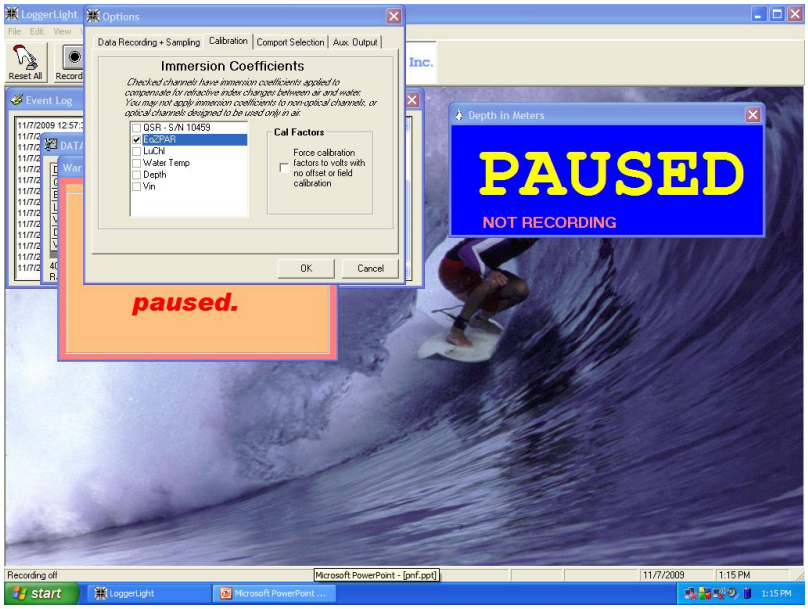
NOTE: This calibration is stored in the computer only, NOT in the PNF. It is used as a field adjustment to the baseline.



3. Go to “Edit” → “Options” (CTRL “O”) and check the “Rapid sampling, maximum rate, no statistics” box under the Data Recording + Sampling tab. This will ensure the highest resolution of data for the profile cast.



4. Go to “Edit” → “Options” (CTRL “O”) and check that the UW sensor (EoZPAR) box is checked in the “Immersion coefficient” section under the Calibration tab.



5. Pressing “view” → “depth” will show the large numerical display of instrument depth (in the image above it says “paused”). “Data” and “Log” should also be checked so you can view these on the screen.

Deployment

1. The PNF is always cast in an outside hole to minimize shading effects (i.e., Weatherport shading). The sampling hole must be covered with a tarp to minimize direct sunlight entering the hole.
2. The surface PAR sensor should be placed away from the hole to eliminate possible shading.
3. Position the PNF over the center of the hole and lower the PNF until the downwelling PAR sensor is covered by water. Allow the PNF to cool and the pressure transducer to stabilize. (about 2 Mins)
4. Raise PNF into the air and start recording "File" → "Start Recording."
5. Lower the PNF back into the water and slowly lower (0.5 m sec^{-1}) to the desired depth, pausing at this depth for 10 seconds (take note of the depth).
6. Stop the cast at this depth by going to "File" → "Close."
7. The file will automatically save in the folder you created on the c: drive ("File" → "Save As")
8. View the file to ensure the instrument was working properly.

Post-Deployment

1. Record 30-60 seconds of dark with UW sensor covered in order to keep a record of dark voltages.
2. If desired, save the Log File by going to "File" → "Save Log File"

Sensors

The Teflon may become dirty during normal use. Clean gently with warm water, soap or a mild solvent such as alcohol using a soft tissue or towel. Be gentle -- do not twist the collector. Do not use acids, abrasive cleaners or brushes, as these will mar the surface and invalidate the calibration. Should the collector become disturbed, damaged or heavily soiled, return the instrument to the factory for service and recalibration. Before use you can wipe the collector, using a soft, clean, lint-free cloth.

Seabird 25 Conductivity, Temperature and Depth Instrument

(using new Seasave V7 software. For method using old version of Seasave (V5.39), see appendix of Previously Used Methods)

General Discussion

Materials

Seabird 25 CTD (80 m)
Downloading cable
PC laptop computer
1% Triton-X solution
DIW water
60 ml syringe and tubing to connect to the pump outlet

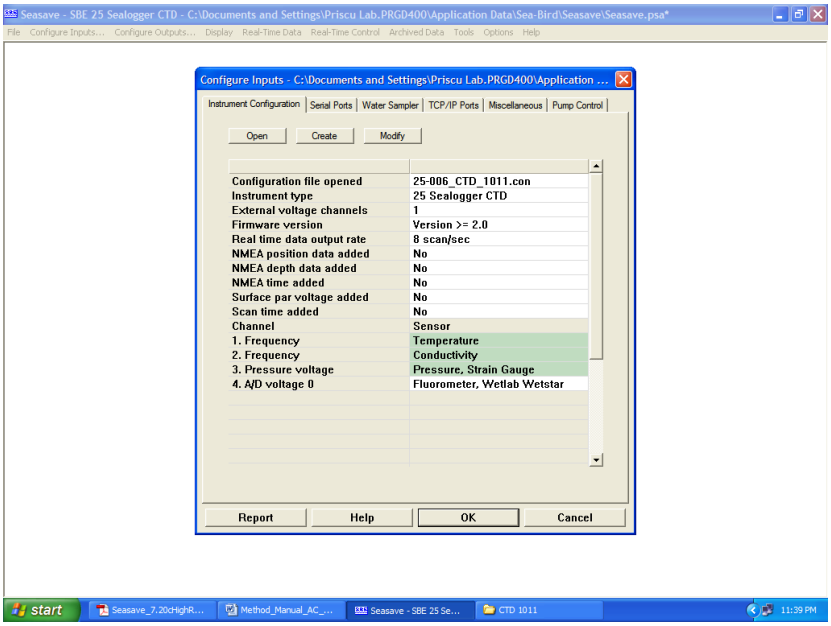
Procedure

Instrument Calibration (SeaSave)

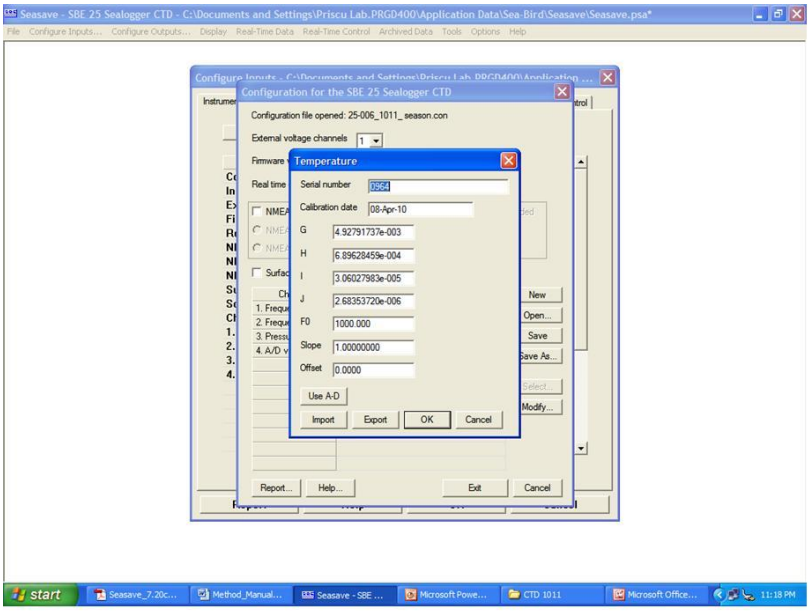
1. Install the current version of Windows Seasoft data acquisition software onto the laptop computer. **SeaSave V7** is used to view and change .con file data, **SeaTerm** is used to communicate with the CTD, do the casts, and download the data, **SeaSave V7** is used to view the data, and **SBE Data Processing** is used to process the data (convert .hex files).

2. Configure the data acquisition software based on the current calibration constants provided by Sea-Bird Electronics. Every year the manufacturer sends a paper and electronic (on a CD) copy of the calibration values, and an electronic copy (on a CD) of the .con file containing these calibration values. Save the current .con file to the computer from the CD and view it in SeaSave to check the values. In SeaSave, click on "configure inputs" → "instrument configuration" then "open" and choose the appropriate .con file. If you don't have a .con file, you can modify an existing file with the values from the paper copy. Enter the values as illustrated below:

- Enter "> than highest" for firmware (Gary Morast from SeaBird said that the Firmware version is important to have correct. In 0708 it was 4.02)
- "8 scans per second" for Data Output Rate (this is user configurable: When our CTD come back from the manufacturer, it is set to store data at 1 scan per second; we will change this to 8 scans per second using SeaTerm (see below)).



Check the Temperature, Conductivity (Set the Cell Constant = 2000, and the Series Resistance = 300), Pressure and Fluorometer values, or enter them from the paper copies of the calibration reports if you are not using the current .con file. If you have selected the current .con file, these values should match the values in the paper copy. To do this, double click on the parameter you want to check, or click on modify. Below are the 1011 calibration coefficients for the Temperature Sensor.



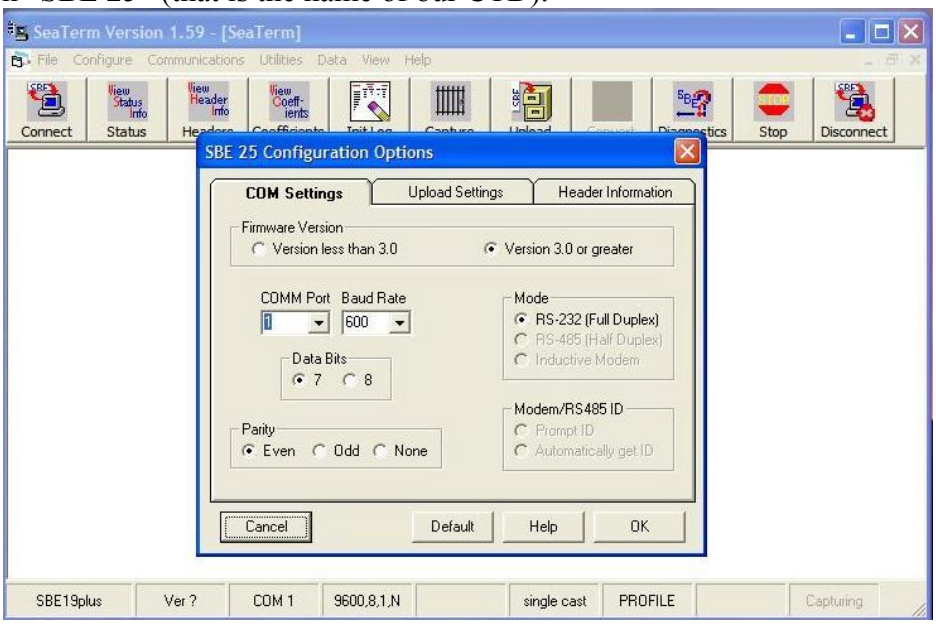
Take note of the serial numbers in the .con file for the temperature and conductivity sensors as we will make sure later that the serial numbers in SeaTerm match these.

Save the configuration file. The .con file is used for data processing (conversion of .hex files) with SBE Data Processing, and for viewing data in SeaSave. SeaSave and SBE Data Processing will not interpret the data correctly without the correct .con file.

When exiting the SeaSave program, if you save changes to the program file when it asks, the program will remember the .con file you used in the future.

Instrument Setup (SeaTerm v1.59)

1. Before using SeaTerm to communicate with the CTD or upload data from the CTD, the program must have information about the CTD hardware configuration and about the computer. To set these parameters, open the SeaTerm program, go to "configure" and click on "SBE 25" (that is the name of our CTD).



Information about the CTD's configuration came with the original instrument purchase:

<u>COM settings (must match CTDs configuration sheet):</u>

a. Firmware: > 3.0 (updated to 4.02 in 0708) **(must be correct according to Gary Morast of SeaBird)**
b. Comm port – 2 (this was assigned to the serial to USB converter in 1112 season).
c. Baud Rate – 600 (the program cycles through the baud rates until it finds the one it can use to connect. You can either put 600 and let it find the right one, or put the one it uses (usually 4800) to start out with).
d. Data Bits – 7
e. Parity – even

Upload settings:

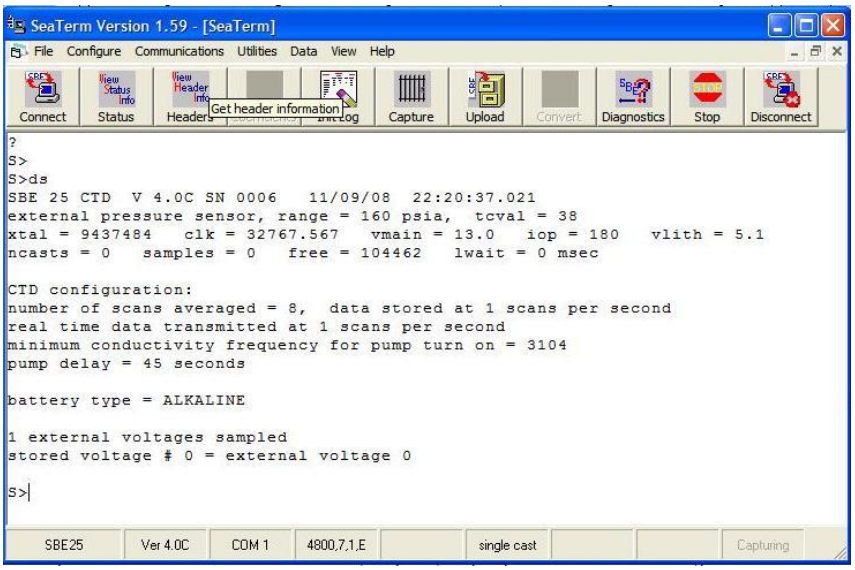
a. Data Upload Baud Rate – 9600
b. Upload Data: 1 cast (set as desired)
c. SBE 3 - Temperature Sensor Serial Number: 0964 **(must match .con file)**
d. SBE 4 - Conductivity Sensor Serial Number: 2396w **(must match .con file)**

Header Information (set as desired):

a. Header: Prompt for Header Information

When done, click OK and SeaTerm will save the settings in a SEATERM.ini file. SEATERM will not upload data correctly without a properly configured SEATERM.ini file. (If the program saves the settings, it has created the .ini file in the root directory; you don't actually have to do anything with the .ini file).

Click "Connect" to communicate with the CTD
Click "Status" to get information about the CTD.



When the CTD comes back from the factory every year, it is set to turn on at a salinity meant for using it in seawater. This parameter is called the "minimum conductivity frequency for pump turn on." Since we are using it in the lakes, we need to re-set this value so that the CTD will turn on in relatively freshwater. After you have established communications (above), check the minimum conductivity frequency for pump turn on in the CTD configuration list that appears. Generally it will be ~ 3000 Hz from the factory. To set the new pump start frequency:

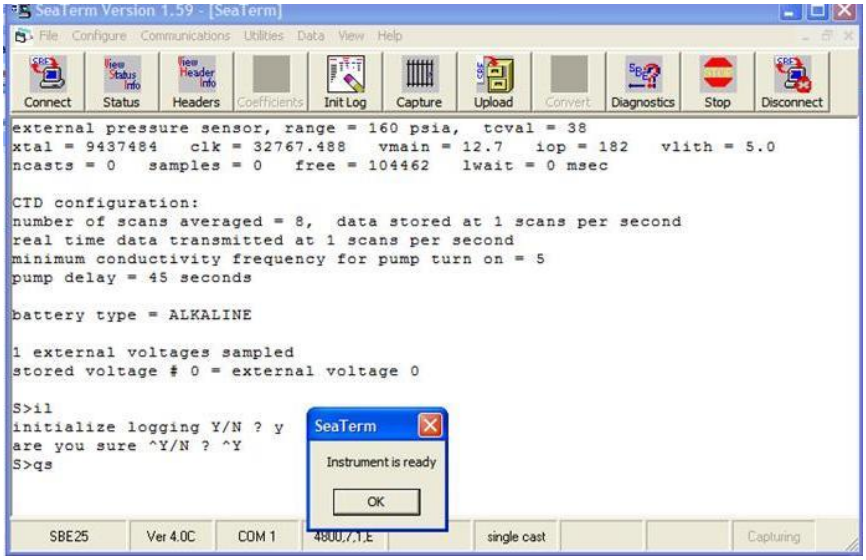
At the s> or #s> prompt, type cc
type Y when it asks Y/N
type CTRL Y when it asks CTRL Y/N
Press enter to keep the rest of the values as they are, but change the pump start frequency to 5.

When the CTD comes back from the factory every year, it is set to a Real Time Data Output (or Transmission) Rate and Data Storage Rate of 1 scan per second. We need to change these to 8 scans per second. (The con file generally has the Real Time Data Output Rate set to 8 scans per second. This will have been checked in SeaSave already). After you have established communications, check the Data Storage Rate and the Real Time Data Transmission Rate in the CTD configuration list that appears (see above). Generally they will have been set to 1 at the factory. To set the new rates:

At the s> prompt, type cc
type Y when it asks Y/N
type CTRL Y when it asks CTRL Y/N
Press enter to keep the rest of the values as they are, but change the Internal Data Storage Rate and the Real Time Data Output Rate to 8.

Data Collection (SeaTerm)

1. Before sampling, the SBE 25 CTD must be initialized to clear memory modules. Connect the SBE CTD to the computer and launch the SeaTerm communication program. Press "Connect" to communicate with the CTD, and "Status" to get information about the CTD. Following recognition, press "Init Log" to initialize logging, answer YES to the questions.



Disconnect the cable from the CTD and replace the protective plug. The SBE 25 is now ready for deployment. "qs" refers to quiet or quiescient state. The CTD must be in this state for 2 minutes before the pump turn on switch will work. You can command "qs" in SeaTerm (it automatically did this above), or disconnect the CTD and be sure to wait 2 minutes until turning on the pump switch.

2. Typically the SBE 25 CTD is cast in the Weatherport sampling hole, because it does not measure natural fluorescence or PAR which is influenced by the shading effect from the Weatherport. Remove all of the ice from the hole, **Do Not** allow ice crystals to touch the probes. Attach the CTD frame to the winch cable. Remove the red cap. Use a 60 ml syringe to flush the pumping system and sensors with a 1% Triton-X solution.

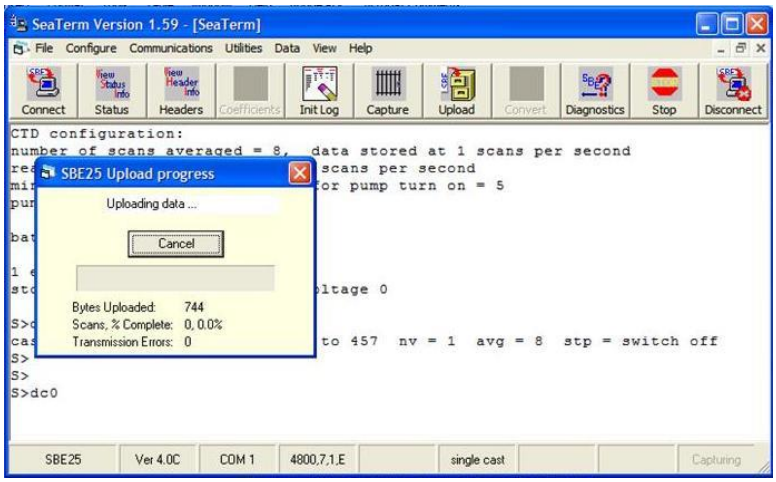
3. Position the CTD over the center of the hole and turn the unit on. Immediately lower the CTD into the water up to the O ring where the frame is attached to the cable. Allow the CTD to thoroughly flush water through the system (>60 sec – it takes 45 second for the pump to turn on once it is put in the water). The CTD may have to be raised out of the water enough to see that the pump is working (it should be pumping a constant stream of water out ***). The pump will only turn on at the salinity you entered in step 2, Instrument Set-up. Once you ensure that the pump is working, lower the CTD to the O ring again, and let it sit for a minute. This depth is ~ 0.8m from the pressure sensor, and will be used to correct the pressure sensor to the correct depth during data processing. This is very important as the pressure sensor is not always accurate!!

*** If there is not a constant stream of water coming from the pump when you check it, the hole in the bleed valve hole at the top of the "Y" fitting at the top of the CTD may be clogged. Clean it out with the 0.016" OD wire that should have been supplied by SeaBird after calibration. When the CTD is submersed, a steady stream of bubbles should come from the hole for at least 5 seconds. Pliers may be needed to get the wire through the hole.

4. Slowly lower the CTD (1 m sec^{-1}) to the desired depth ~2 m from the bottom of the lake **(Do Not** allow the CTD to hit the bottom of the lake), stop for 10 seconds, then slowly retrieve at the same rate. Remove the CTD from the water and immediately turn the instrument off. **Note**: The CTD must be turned off to save each cast. Following data collection, turn the instrument on and lower to 1 m to thoroughly rinse the instrument, remove and place in carrier. Flush the pumping system with DI water after use.

Downloading (SeaTerm)

1. Connect the SBE 25 CTD to the computer and launch the SeaTerm program. Press "Connect" to establish communications. Once communications are established, press "Upload" to Upload data. **Note**: The software may be configured to dump specific casts or all, and to include data headers. Name the file accordingly (i.e., Lake code, date), and save to the desired directory. Every time the pump is turned on and off there is a new cast saved. Therefore, when downloading the data the first cast is 0, second is 1, etc. The multiple casts can be downloaded, but they must be specified when you "upload" the data in SeaTerm.

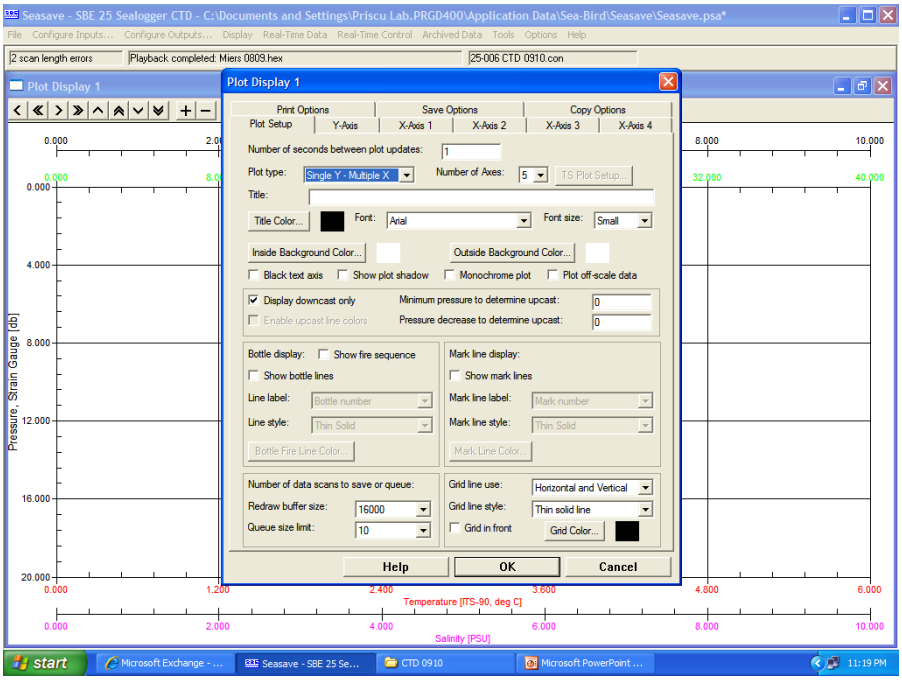


2. View the data to make sure the CTD was operating properly using SeaSave. You can view a plot, or view a readout of the data.

To view a readout of the data, go to "Display" → "Add new fixed display window." Add parameters you want to view (Pressure (db), Temp (ITS-90, °C), Salinity (PSU), Fluorescence WetLab Wetstar (mg/m3), Conductivity (S/m), Density (kg/m3)) . Then go to "Archived Data → Start," select data file and .con file, change number of scans to skip over at start to ZERO (or however many scans you want to skip over), and press start.

To view a plot of the data, go to "Display → "Add new plot display window."

Under the "Plot Setup" tab make the following selections:
Plot Type: Single Y – Multiple X
Number of Axes = 5



Under the "Y-Axis" tab make the following selections:

Set up the y-axis with Pressure (db)

Under the "X-Axis" tabs make the following selections:

Set up the x-axes with:

Temp (ITS-90, °C)
Salinity (PSU)
Fluorescence (WetLab Wetstar (mg/m3)
Conductivity (S/m)

Set up an appropriate range for each parameter for the lake which you are plotting. Make any changes to color, etc desired.

To save graph set-up, close set-up window, right click on graph, and "Export Display Settings (.dsa file)."

To re-enter plot set-up screen, open graph and click on "Display → Modify," or right click on graph and click on Modify.

To open a dsa file that was already set up, Display → "Import Display Settings (.dsa file).

To draw the graph, go to "Archived Data → Start," select data file and .con file, change number of scans to skip over at start to ZERO (or however many scans you want to skip over), and press start.

Note: In past years, problems with data acquisition have occurred when cable connections allowed water to infiltrate and corrode the connections. If data appears to be chaotic, remove all cable connections and thoroughly dry the connections. Lube the connectors with stopcock grease and securely tighten the cables.

CTD Data Conversion from .hex files – SBE Data Processing
SBE tech help – Gary Morast (425) 643-9866

June 2010:
We began using the new version of SBE Data Processing, "Version 7.20d, 2010," in June 2010 for the 0910 data. This version is able to calculate density and salinity to the deep depths of Lake Bonney, and any other lakes with high density and salinity water. It also calculates conductivity with greater resolution in the lower depths of Lake Bonney. Prior to this we were using version 5.25.

Sept 2013:
We began using the new version of SBE Data Processing, "Version 7.23.1" in Sept 2013 for the 1213 data. The revisions listed online that applied to the SBE25 CTD were:

- Derive Teos-10 module added (John decided we should stick with the original (PSS-78) derivations).
- Now ignores a 'w' in conductivity sensor serial number in configuration file (indicating wide range conductivity calibration) when checking against serial number in data file. Previously, it would give an error message saying that serial numbers did not match.
- Add selection of units for calculating and outputting Specific Conductance.

To convert the .hex files, you will need to use the SBE Data Processing program.
Programs – Sea-Bird – SBE Data Processing Win 32
You will also need the .con file for that season
It helps to put all the hex files in 1 folder.

1. Set up configuration file
    a. Configure – SBE 25 – you can look at the configuration (.con or .xmlcon) file. Click open and open your .con file. Go into each parameter and make sure the coefficients are correct from the configuration file (disk or printout).
    b. Exit when done

2. Run – Data Conversion
    a. Tab: File set-up
        i. Program set-up file (.psu or .psa) – default (use default file) (you can use any psu file, it is just saving what parameters you set up during your conversions, but you will check these anyway).
        ii. Instrument configuration file – select configuration file for that year (.con)
        iii. Input directory – the program will change the folder depending on where you get your files.
        iv. Input files – select all to convert (.hex) – it is best to put all the files in 1 folder so you can easily select them all at once
        v. Output directory – default or pick your own; the program may change the folder depending on where you get your files.
    b. Tab: Data set-up
        i. In the 7.23.1 version there is a box to click for "Process scans to end of file" – I left it clicked
        ii. Scans to skip over – don't skip over any now but if CTD went slow may need to
        iii. Output format – ASCII
        iv. Convert data from – downcast
        v. Create file types – CNV
        vi. I did not click "Merge separate header file" which is new in the 7.23.1 version – talked to SeaBird tech help and they said not to click it unless we have something else we want to include with header.
    c. Tab: Data set-up - Select Output Variables
        i. Pressure (db)
        ii. Conductivity (mS/cm)
        iii. Temp – ITS-90 (C)
        iv. Fluorescence – Wetlab Wetstar (mg/m3)
        v. Scan count
    d. Tab: Data set-up
        i. I left the default ("Instrument's time stamp) for "Source for start time in output .cnv header" which is new in the 7.23.1 version.

e. Tab: Miscellaneous: latitude when NMEA not available: 77 (doesn't cause a difference in depth)
f. Tab: Header view
    i. Tells about each file – stuff is manually entered for each cast – put in date, lake, weather, time (takes from computer so check). I don't usually change anything on this page.
g. Tab: Go back to file set-up
h. START PROCESS – (if you get an error because the temp serial number in the header and con file don't match, you can change it in the con file to match.)
i. Exit once done (saved changes to .psu or .psa file)

3. Run – Bin Average
    a. Tab: File set-up
        i. Program set up file – same as before
        ii. Input directory – same as before
        iii. Input files – select all (.cnv)
        iv. Output directory – same as before
    b. Tab: Data set-up
        i. Bin type – pressure, no interpolation
        ii. Bin size - 0.1
        iii. Click box to include # of scans per bin (I left box clicked to exclude bad scans)
        iv. Scans to skip over – 0
        v. Cast to process – downcast
        vi. Include surface bin, 0, .1, 0 – no (if include, will put #s in first bin line, but rest of values below the first bin line are the same whether surface bin is included or not).
    c. Tab: Header view – tells info
    d. Tab: Go back to File set-up
    e. START PROCESS – overwrite .cnv – yes to all
    f. Exit – save changes

f. Run – Derive (may say created default file – say ok)
    a. Tab: File Set-up
        i. Program set-up file – default
        ii. Instrument configuration file – select file for this year
        iii. Input directory – same as above
        iv. Input files – choose all (.cnv)
        v. Output directory – same as above
    b. Tab: Data set-up
        i. Select derived variables (secondary derivations using formulas)
            1. Density (kg/m3)
            2. Density (sigma-theta, kg/m3)
            3. salinity (PSU)

4. Depth - freshwater (m)

c. Tab: Miscellaneous: latitude when NMEA not available: 77 (doesn't cause a difference in depth)
d. Tab: Header view – just info
e. Tab: Go back to File set-up
f. START PROCESS – overwrite all .cnv files - yes (adds on new data)
g. Exit – save changes to psa or psu file - yes

g. Run – Buoyancy (may say created default file – say ok)
   a. Tab: File set-up – same as above – select all .cnv files
   b. Tab: Data set-up
      i. buoyance variable: Latitude
      ii. Latitude: - 77 (the program takes an absolute value for latitude)
      iii. window size: 3
      iv. units
         1. buoyancy frequency [N^2, rad^2/s^2]
         2. stability, E (rad2/m)
   c. Tab: Header view – just info
   d. Tab: Go back to File set-up
   e. START PROCESS – overwrite all .cnv files - yes (adds on new data)
   f. Exit – save changes to psa or psu file - yes

h. Run – ASCII out (separates header into different file) – (creates default file)
   a. Tab: File set-up – same as above - select all .cnv files
   b. Tab: Data set-up – check top 3 boxes
      i. Lines – 60
      ii. Label columns – top of the file
      iii. Column separator – space
      iv. Julian days – julian days (this was in the 7.20d version) - In version 7.23.1, there is a box to click on for "Select Time Conversion Formats" – I left them all as "Do not convert"
      v. Other 2 boxes blank (this was in the 7.20d version) - In version 7.23.1 there is an "Add first column" box – I left it blank)
      vi. Replace bad flag – don't check (makes no difference if check box)
      vii. Select output variables (all should be checked)
   c. Tab: Header view – just info
   d. Tab: Go back to File set-up
   e. START PROCESS
   f. Exit – save changes to psa or psu file - yes

The files are now in the folder you chose as the Output Directory. You have CNV, ACSCII (Wordpad) and HDR files. Use ASCII files because these have headers, CNV do not. Open Excel – open Word Pad (ASCII) files – save as Excel.

bbe Fluoroprobe – Underwater Spectral Fluorometer

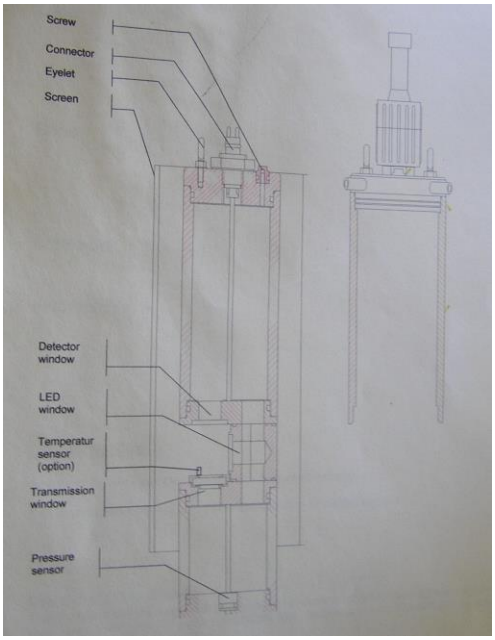
General Discussion

The bbe Fluoroprobe is a submersible spectrofluorometer which differentiates the four major groups of phytoplankton in the lakes (cyanobacteria, Chlorophyta, Chrysophyta, Cryptophyta) based on the chlorophyll-a fluorescence excitation spectra of the light harvesting apparatus. Algal chlorophyll-a is excited with light of five LEDs (emission wavelength 450 nm, 525 nm, 570 nm, 590 nm, 610 nm). Dissolved yellow substances measured at 370 nm are used to correct for background fluorescence in the algal algorithms. Algal group concentrations are given in µg chl-a/L water sample. (This instrument was new in 0405 season).

Materials

bbe Fluoroprobe and cable
PC laptop computer
sealant
autoplug

The Fluoroprobe program version 1.8.4 was used until the 11-12 season, and the below methods are for that version. A new laptop was purchased for this season which would no longer run version 1.8.4. We started using the newest version (2.2.6.2) during this season. Any changes with the new program are noted below.



(Figure 1)

Procedure

Instrument Calibration

Validate communication between the probe and the computer by hooking the probe cable up to the computer cable. You can also save the probe battery by using the combo cable that plugs into the main power, the probe and the computer.

- Test connection: ctrl+T (or Probe→Test connection)
- Test voltage battery voltage: Probe →Battery voltage --if voltage is around 11V, recharge the battery with the charger overnight.
- Set the date and time on the Fluoroprobe by going to Probe → Set date/time. If the probe has been sent to the manufacturer for calibration, the time may be set to a different time zone.

1. Sample Temperature - 2-point Calibration

(For the 2 point calibration, water of 2 different temperatures, >10° C apart, and preferably covering the common range of the samples, are needed. Since the temperature range of our samples is not very large and is typically very low, it is difficult to cover the range of our samples). During the calibration measurement the Fluoroprobe has to be moved permanently (I think this is supposed to be "continuously"):

- Go to File → Get data and parameters → Parameters only - the computer will pull the parameters stored in the Fluoroprobe to the computer.
- Go to Calibration → Sample Temperature (Figure 2).
- Place the probe in a bucket of ice cold water measured with a thermometer, such that the water covers the temperature sensor (Figure 1).
- Enter the temperature of the water as read on the thermometer in the "first calibration point" window.
- Allow the current probe temperature as read in the display window to stabilize.
- Press Hold next to the "first calibration point" window.
- Place the probe in a bucket of room temperature water measured with a thermometer, such that the water covers the temperature sensor (Figure 1).
- Enter the temperature of the water as read on the thermometer in the "second calibration point" window.
- Allow the current probe temperature as read in the display window to stabilize.
- Press Hold next to the "second calibration point" window.
- Press Apply to send the results of the calibration (new offset and gradient) to the FluoroProbe. (Next time you get parameters from the Fluoroprobe, the program will pull these calibrations from the instrument).
- Press X to exit this display window.

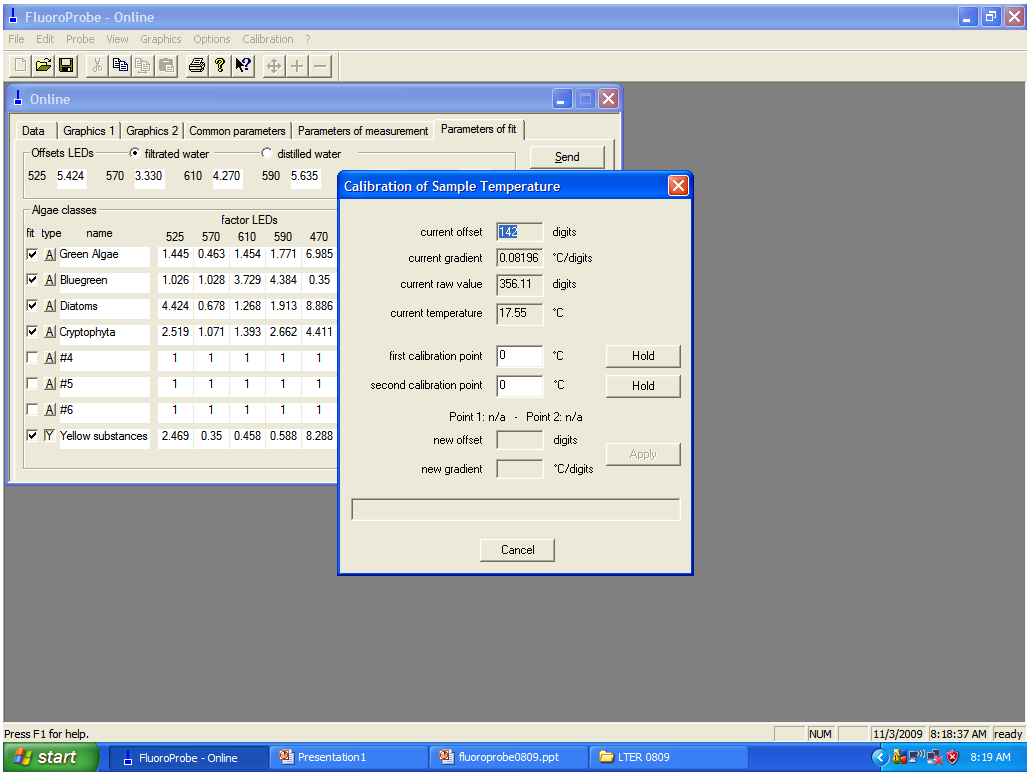


Figure 2.

2. Transmission Calibration

- Go to Probe → Get data and parameters → Parameters only - the computer will pull the parameters stored in the fluoroprobe to the computer (this will already have been done if doing the above calibration).
- Go to Calibration → Transmission (Figure 3).
- Press Start under the offset section of the display window.
- The probe will instruct the user to darken the detector window – Remove the protective case surrounding the fluoroprobe, and darken the detector window with a black cloth or piece of paper (Figure 1).
- Follow the instruction to calibrate the offset – the probe will take 10 measurements.

  **With the new 2.2.6.2 program that we began using during the 11-12 season, pressing the start button triggers a continuous transmission measurement. The readings are then shown in the scrolling window to the right. If you press the button "apply" the new offset is taken and shown in the small frame under the old offset. The readings in the window to the right continue flowing - that is normal. Put the fluoroprobe into water then and start the calibration with the start button related to the gradient calibration. The readings reach somewhere around 100%. By pressing the "apply" button a new value for the gradient is taken - like before. (I got this information from Tim Doyle (our US Fluoroprobe rep), because it was still described in the new manual as it was in the old manual version). **

- Press Apply in the offset section of the display window (sends the results of the offset calibration to the FluoroProbe. Next time you get parameters from the Fluoroprobe, the program will pull the new offset calibration from the probe.)
- Press Start under the gradient section of the display window
- The probe will instruct the user to place the probe in Distilled water - Place the probe in a bucket of distilled (use DI) water such that the water covers the detector window.
- Follow the instruction to calibrate the gradient – the probe will take 1 measurement.
- Press Apply in the gradient section of the display window (sends the results of the gradient calibration to the FluoroProbe. Next time you get parameters from the Fluoroprobe, the program will pull the new gradient calibration from the probe.)
- Press X to exit this display window.

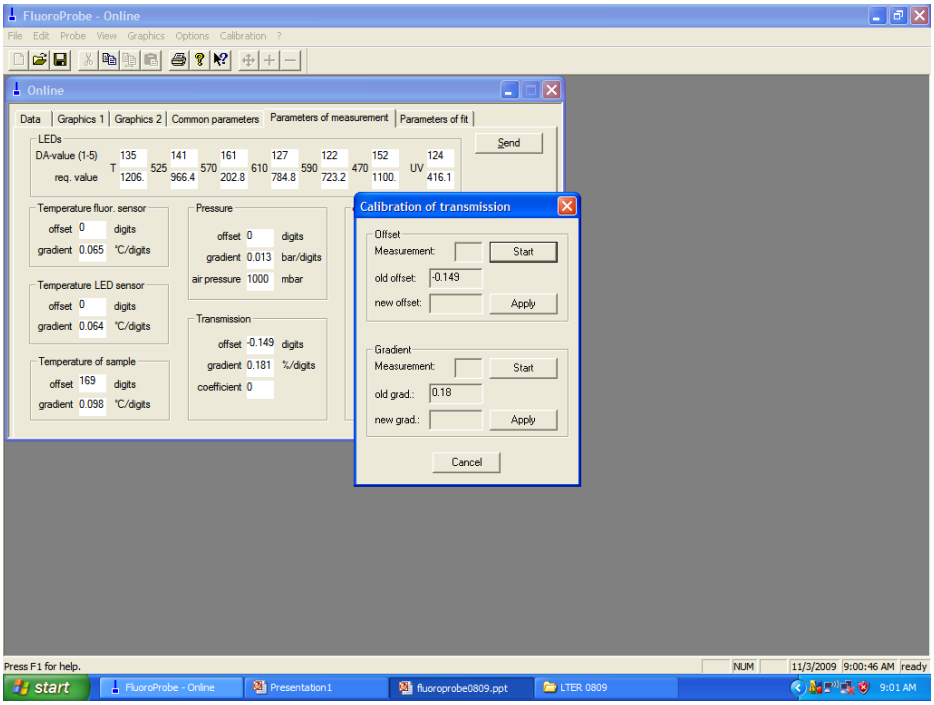


Figure 3.

3. Distilled Water Offset Calibration (ultrafiltrated offset calibration is not needed for Limno Sampling – the ultrafiltrated offset is only used when the determination of yellow substances is turned off; the distilled offset is used when the determination of yellow substance is turned on)

- Place the probe in a bucket of distilled (use DI) water such that the water covers the sampling window (need at least 30 cm of clearance) (Figure 1).
- Avoid artificial light during calibration, especially fluorescent lamps! Therefore, shut off all lights.
- Go to Probe → Get data and parameters → Parameters only - the computer will pull the parameters stored in the fluoroprobe to the computer (this will already have been done if doing all the above calibrations).

- Go to Calibration → Offsets (distilled) (Figure 4).
- Press Start – the probe will take 10 measurements and display the new distilled water offsets.
- The transmission will be displayed following the calibration - the transmission should read approximately 100%. If it is not, re-do calibration of the transmission.
- Press Apply (sends the results of the calibration to the FluoroProbe. Next time you get parameters from the Fluoroprobe, the program will pull the new Distilled water offsets from the probe).
- Choose OK in response to probe warning.

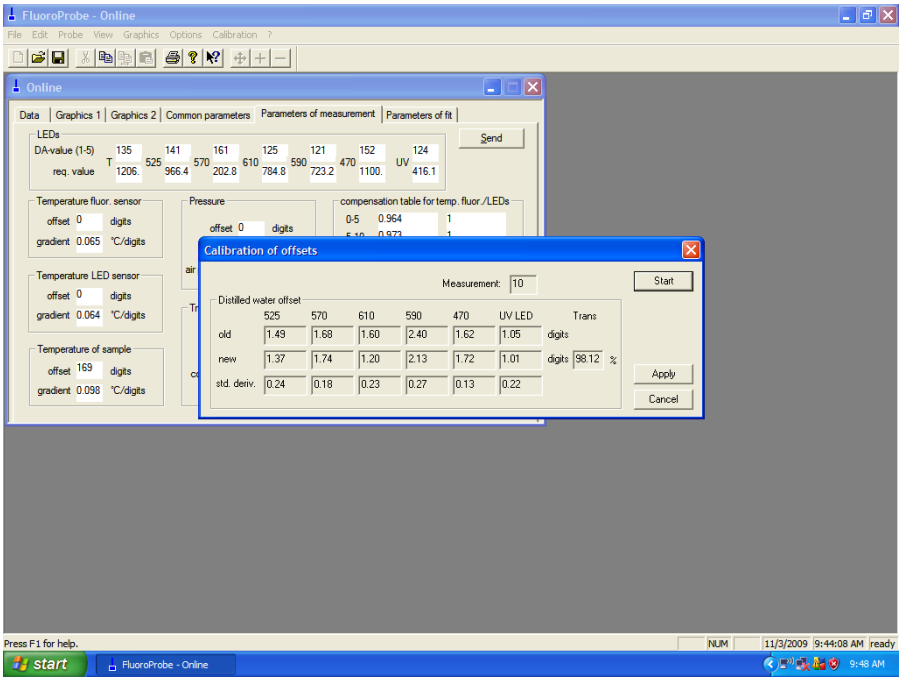
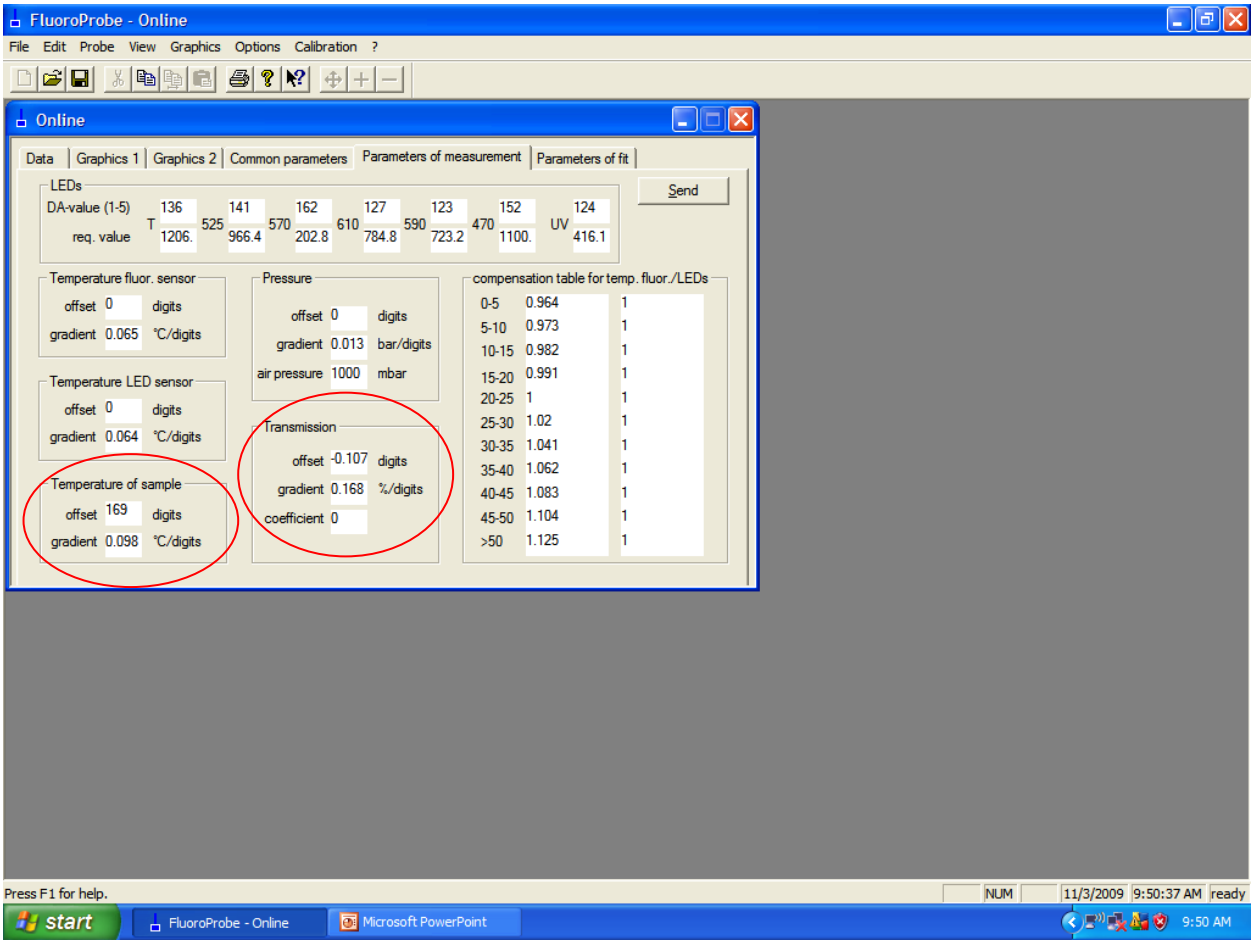


Figure 4.

Check the Calibrations

- The calibrations for Sample Temperature, Transmission and Distilled water offset can be checked after calibration, and each time you operate the Fluoroprobe.
- Once the probe is connected to the computer (if you have just done the calibrations, you will already be connected), go to File → Get data and parameters → Parameters only - the computer will pull the parameters stored in the fluoroprobe to the computer.
- The Sample Temperature and Transmission offset and gradient calibrations will be displayed in the "Parameters of Measurement" tab (Figure 5).
- The Distilled Water Offsets will be displayed in the "Parameters of Fit" tab (Figure 6).


FluoroProbe - Online
File Edit Probe View Graphics Options Calibration ?
Online
Data Graphics 1 Graphics 2 Common parameters Parameters of measurement Parameters of fit
Send
LEDs
DA-value (1-5) 136 141 162 127 123 152 124
req. value T 1206. 525 966.4 570 202.8 610 784.8 590 723.2 470 1100. UV 416.1
Temperature fluor. sensor
offset 0 digits
gradient 0.065 °C/digits
Temperature LED sensor
offset 0 digits
gradient 0.064 °C/digits
Temperature of sample
offset 169 digits
gradient 0.098 °C/digits
Pressure
offset 0 digits
gradient 0.013 bar/digits
air pressure 1000 mbar
Transmission
offset -0.107 digits
gradient 0.168 %/digits
coefficient 0
compensation table for temp. fluor./LEDs
0-5 0.964 1
5-10 0.973 1
10-15 0.982 1
15-20 0.991 1
20-25 1 1
25-30 1.02 1
30-35 1.041 1
35-40 1.062 1
40-45 1.083 1
45-50 1.104 1
>50 1.125 1
Press F1 for help.
NUM 11/3/2009 9:50:37 AM ready
start FluoroProbe - Online Microsoft PowerPoint 9:50 AM
Figure 5.

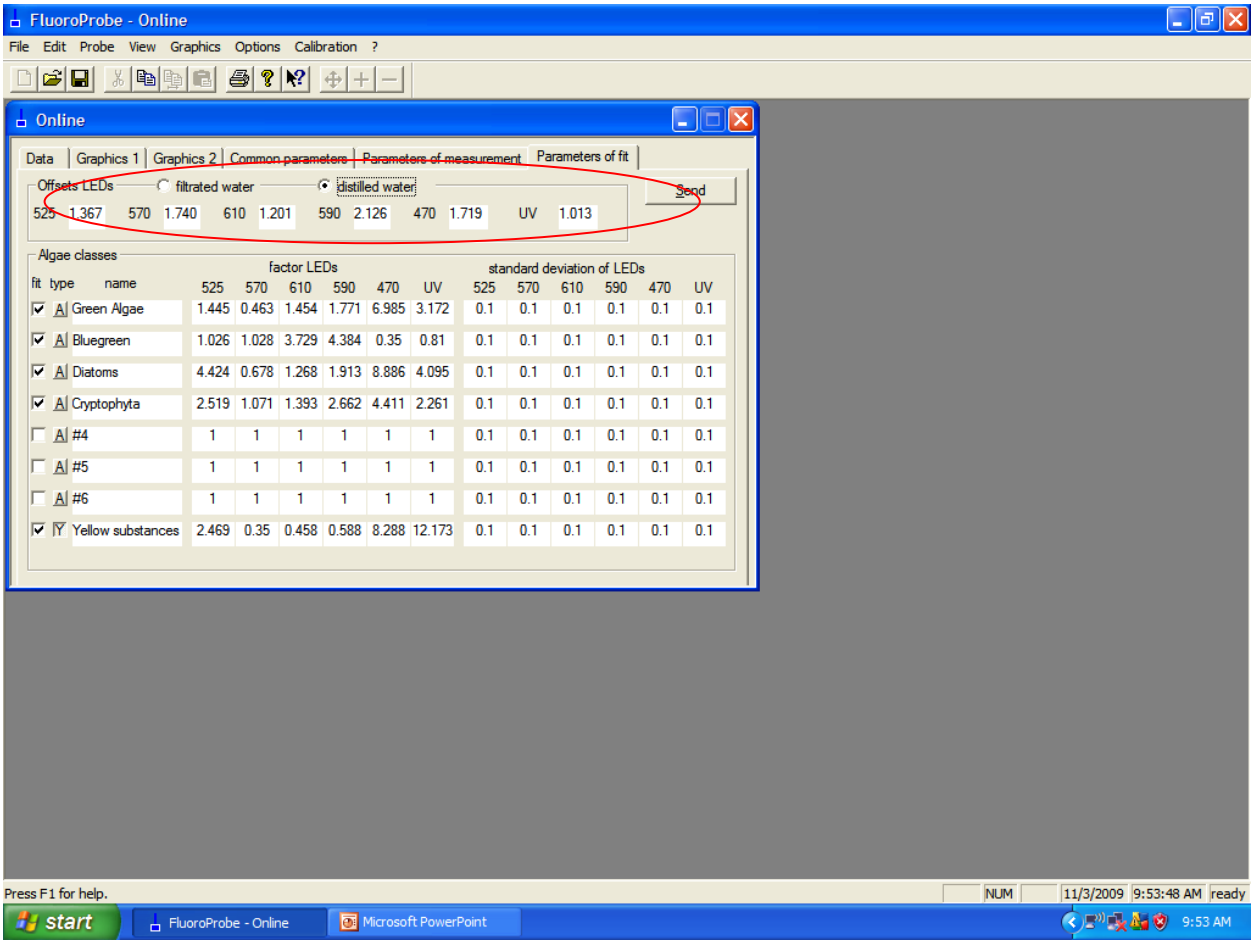


Figure 6.

Data Collection

1. Check parameters in the hut or the polar haven (you will need a computer if programming at the polarhaven):

- Connect probe to computer
- Test connection: ctrl+T (or Probe→Test connection)
- Test voltage battery voltage: Probe →Battery voltage --if voltage is around 11V, recharge the battery with the charger overnight.
- Delete old data stored on the fluoroprobe: Probe → Delete data → type "0"
- Go to Probe → get data/parameters → choose parameters only
- You can check the calibrations for Sample Temperature, Transmission and Distilled Water Offset if desired (see above section on checking the calibrations).
- Check current common probe parameters under the common parameters tab (Figure 7).

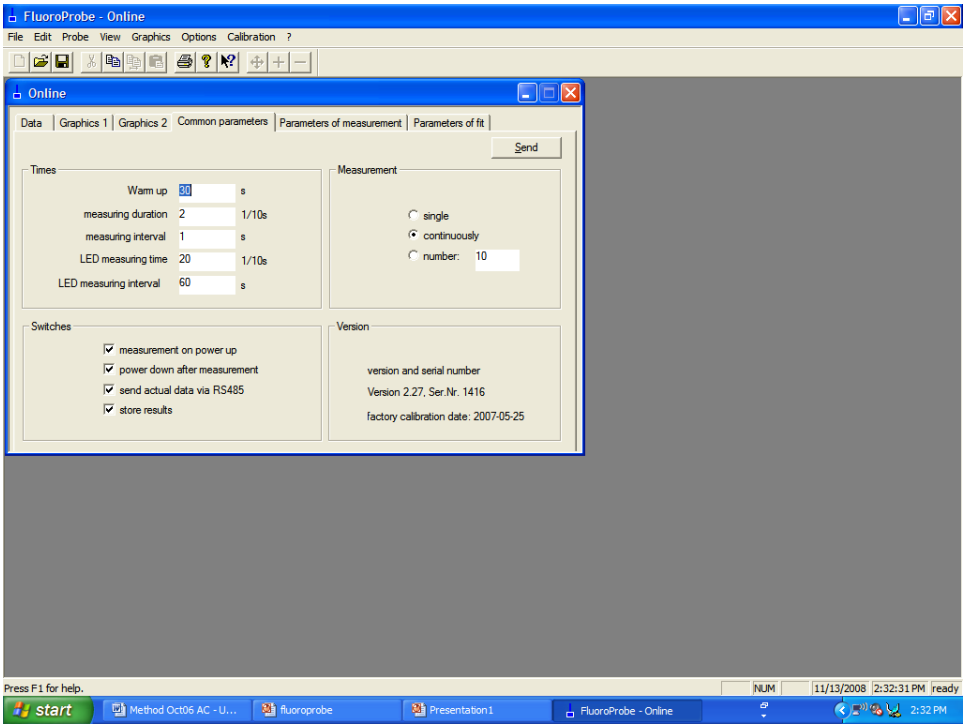


Figure 7.

For Depth Profiles:

Warm up: 30 s
Measuring duration 2 1/10 s (ie. 0.2 s)
Measuring interval 0 s
LED measuring time 20 1/10s
LED measuring interval 60 s
Measurement: continuously
Switches: check all parameters

Click "Send" to send the parameters to the probe if you change them. Next time you connect with the probe they should be the same because it will download the parameters from the probe that were saved.

For Time Course Experiments:

Parameters as above except increase measuring interval to 300 s

2. Set Air Pressure (to get a correct measurement of the depth from the integrated pressure measurement, it is necessary to subtract the air pressure):

- Go to "Probe" – Set Air Pressure (Figure 8).
- A Window will appear displaying the pressure read by the pressure sensor on the probe.
- With the probe above water, click Apply on the average pressure as displayed in the window (note the pressure chosen in your field notebook).

- The air pressure will be set to the new value in the computer, and the pressure you chose will be displayed in the "Parameters of Measurement" tab (Figure 9).

This pressure will be used in the depth calculation by the program once the data are uploaded to the computer after the cast. Since the air pressure is set in the computer, it is best to leave the Fluoroprobe program open during the cast (between starting the cast and uploading the data), and to use the open program with which the air pressure has been set to upload the data from the Fluoroprobe. If the program is closed between casting and uploading the data from the probe, the pressure will need to be reset AND sent to the probe before downloading data from the probe. To do this, enter the correct pressure into the "Parameters of Measurement" screen in the "air pressure" field of the "pressure" section, and click "send" to send the data to the probe. Then upload the data by "getting data and parameters," and the depth will be calculated correctly.

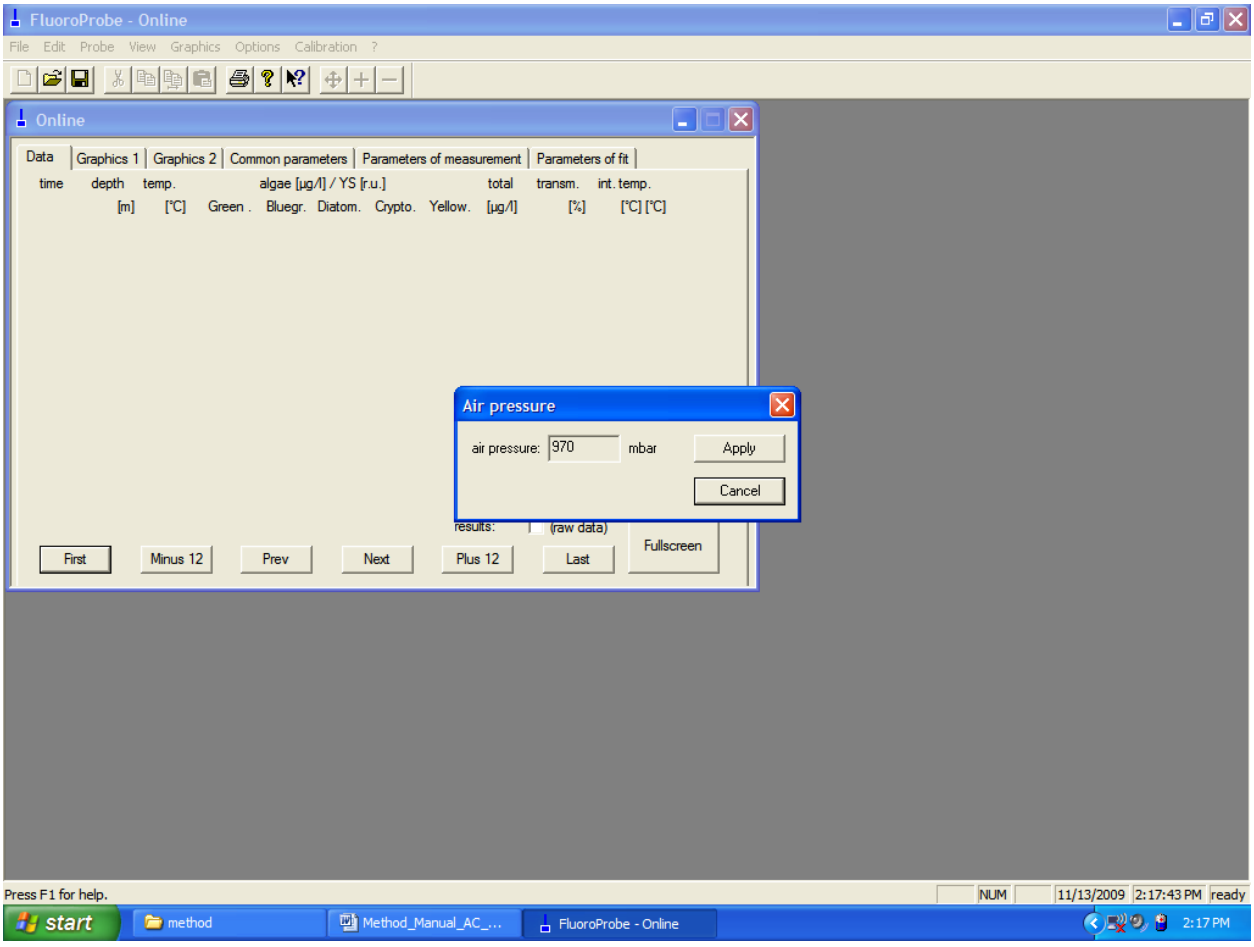


Figure 8.

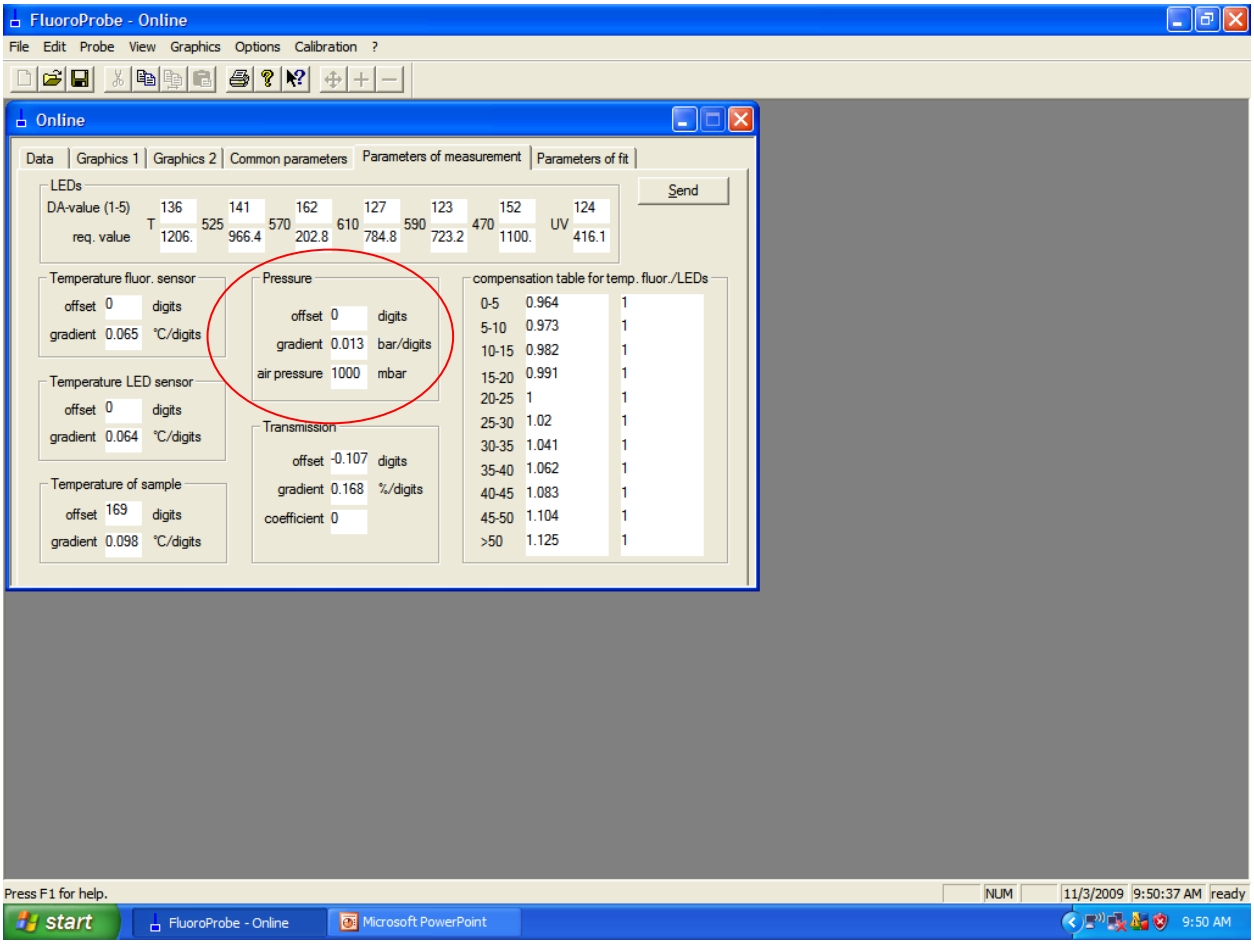


Figure 9.

3. Press F5 to start measurement (or probe → start measurement): The probe will start continuous measurement. The probe will continue recording as long as it is hooked up to the computer (you will see records begin to appear in the "data" tab – Figure 10). Pull the computer plug from the Fluoroprobe to disconnect it from the computer and place on the protective cap. Once you disconnect the cable from the probe, it will stop taking measurements, until you plug in the auto-start plug. Once you plug in the auto start plug, the probe will continue measurements. These will be downloaded to the computer after the cast is done. Don't plug in the auto start plug until you are ready to start the cast because measurements will be taken, and there is a limited amount of space in the probe. The fluoroprobe will hold maximum 1700 data points before it rewrites over previous data.

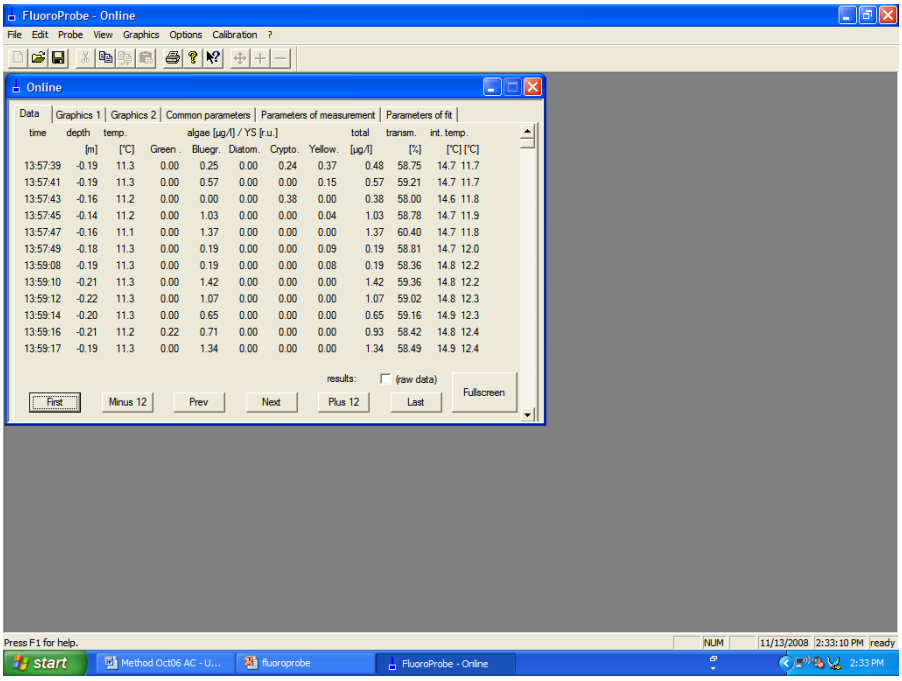


Figure 10.

4. To cast:
   - Remove protective cap; remove dirty/dried sealant and reapply to plastic areas as
   - necessary; reapply sealant to plastic areas of autoplug if necessary.
   - When ready to cast; insert auto start plug (ensure that plug fits tightly and makes a popping sound when it seals).
   - Cast fluoroprobe: lower at a rate of 5 seconds per meter so that each meter has at least 10 data points.

Downloading

- Hook the Fluoroprobe up to the computer
- (If the probe continues recording when it is plugged back into the computer - press F6 to stop the measurement (or Probe → stop measurement).
- Choose Probe → get data parameters → choose data and parameters. This will upload the data and parameters that are stored in the Fluoroprobe from your cast.
- Check that the cast was successful by checking data in the data tab (Figure 10) or the graphics tab (Figure 11).
- Save data on computer by exporting to a text file by clicking on File→Export; this can later be converted to Excel. You can also save the .flp file (File→Save As) if you would like to view the graphics later in the Fluoroprobe program.

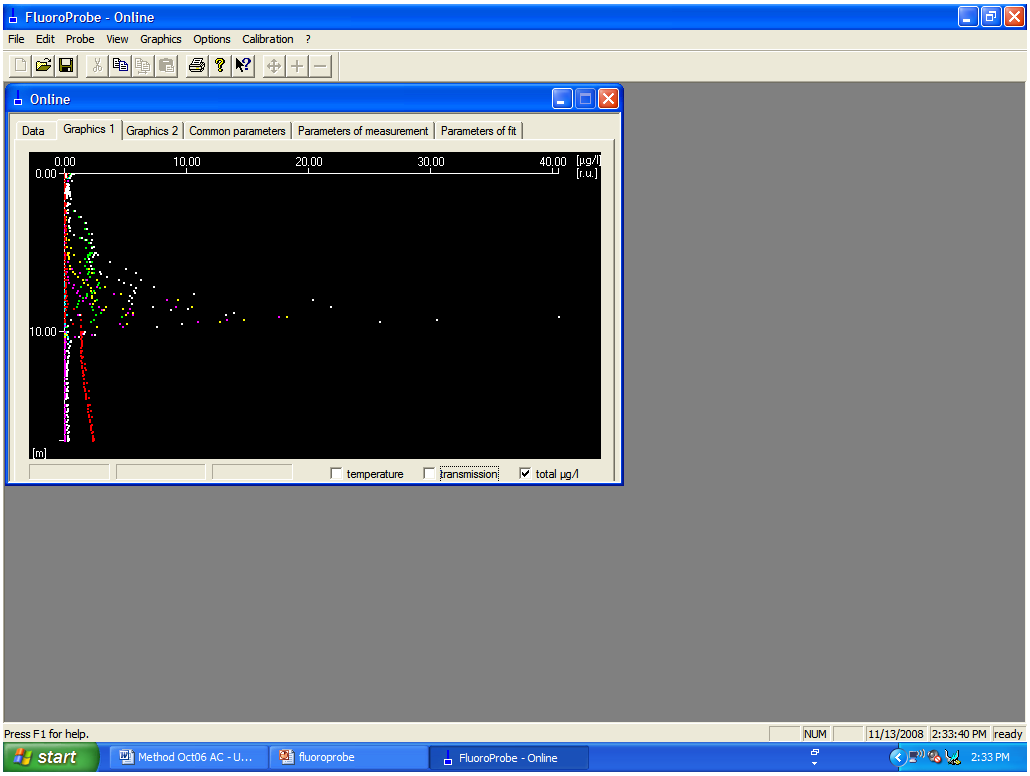


Figure 11.

bbe Fluoroprobe information:
Contact Name: Rudiger Olbrich (engineer)
Phone: ++49 431-380400
Email: Rolbrich@BBE-moldaenke.de
Serial Number: TS-14-16

Optic Stowaway Temperature Logger (Onset Corporation)

Materials
Optic Stowaway Temp Logger
Optis Base Station
Optic Coupler
BoxCar program
Downloading cable

Figure 1.

Procedure
Download the BoxCar program onto the laptop.

Plug the Optic Stowaway temperature logger and the Optic Base Station into opposite sides of the Optic Coupler as shown in Figure 1.

Open the Box Car program and click on “Logger” – “Launch” (Figure 2)

- Set the Interval (Duration) to 5 min.
- Set the Measurement Unit to Temperature C
- Unclick all boxes under Advanced Options

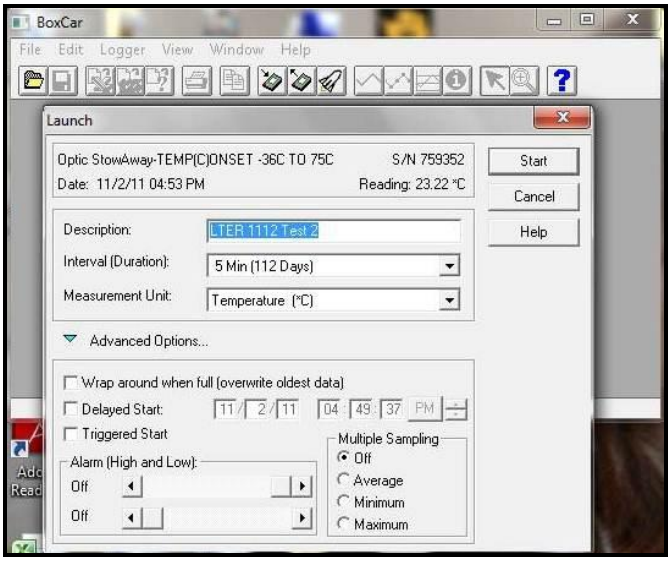


Figure 2.

To retrieve data:

Open the Box Car program and click on “Logger” – “Readout” (Figure 2)

Save the .dtf file. Go to “File” – “Export” and save as an Excel file. It will save it as a text file, and you can open it in Excel. The program will also show you a plot of the temperatures (Figure 3).

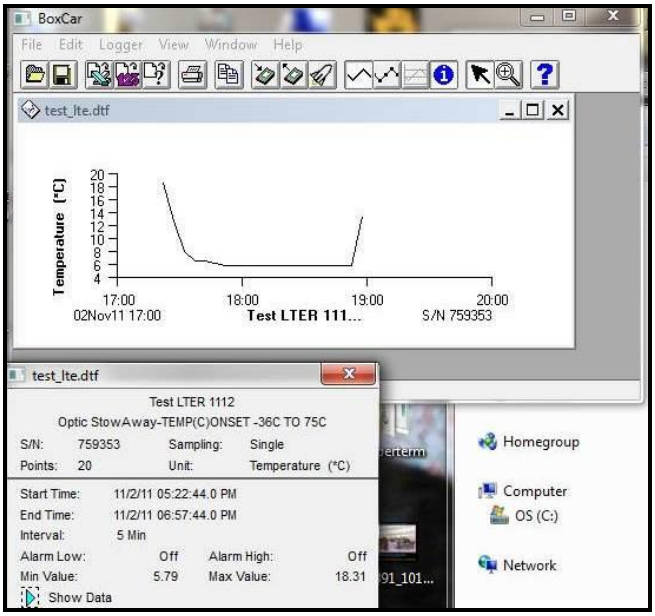


Figure 3.

Sample and Data Submission

Sample Chain of Custody

Chlorophyll-*a*, Anions/Cation, Dissolved Organic Carbon, Particulate Organic Carbon and Nitrogen, and Macronutrient samples are analyzed by Analytical Services each season; the rest of the samples collected are analyzed by Limno Team members, either in McMurdo or upon return to Montana State University. Submission of analytical samples is done through the Analytical Services Team Leader using the Chain of Custody (COC) forms. These forms provide the Analytical Team in the field and the Data Manager with information on all the samples being submitted for analysis. The COC forms also contain information on Limno runs performed each season, on *in-situ* measurements made, and on data which will be submitted to the MCM Database from samples analyzed by the Limno Team. Because information from the COC forms is uploaded into the MCM database, the forms must be filled out completely and accurately by the group submitting the samples. These forms provide a permanent record of all information gathered from each Limno Run for each season. COC forms can be found on the MCM website at http://www.mcmlter.org/restricted/analytical_services.htm.

Data Submission

Data from all analyses and measurements performed during Limno Runs are submitted to the Data Manager to be uploaded into the MCM database. Data must be submitted in specific formats. Data submission formats and instructions can be found on the MCM website at http://www.mcmlter.org/restricted/data_management/limno_submission.htm.

The above sites are part of the restricted access portion of the MCM-LTER website. For access, email the data manager at mcmlter@bprc.mps.ohio-state.edu for the username and password.

West Lake Bonney Sample Volumes (ml)

DEPTH	PPR	DIC	pH	DO	CHL	NUT	DOC	CN	AN/CAT	PHYTO	PP	BAC	TDR	µl ^{14}C	DEPTH
4.5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	220	4.5
5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	220	5
6	600	50	50	50	100	100	100	500	200		500	18		220	6
8	600	50	50	50	100	100	100	500	200		500	18		220	8
10	600	50	50	50	100	100	100	500	200	500	500	18	7.5	220	10
12	600	50	50	50	100	100	100	500	200		500	18		650	12
13	600	50	50	50	100	100	100	500	200	500	500	18	7.5	650	13
14 QC	600	50	50	50	100	100	100	500	200		500	18	7.5	650	14
15	600	50	50	50	100	100	100	500	200		500	18	7.5	650	15
17	600	50	50	50	200	100	100	500	200		500	18	7.5	650	17
20	600	50	50	50	200	100	100	500	200	500	500	18		650	20
22		50	50	50	200	100	100	500	200		500	18			22
25		50	50	50	200	100	100	500	200		500	18	7.5		25
30 QC		50	50	50	200	100	100	500	200		500	18	7.5		30
35		50	50	50	200	100	100	500	200		500	18			35
38		50	50	50	200	100	100	500	200		500	18			38

East Lake Bonney Sample Volumes (ml)

DEPTH	PPR	DIC	pH	DO	CHL	NUT	DOC	CN	AN/CAT	PHYTO	PP	BAC	TDR	µl ^{14}C	DEPTH
4.5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	220	4.5
5	600	50	50	50	100	100	100	500	200		500	18	7.5	220	5
6	600	50	50	50	100	100	100	500	200	500	500	18		220	6
8	600	50	50	50	100	100	100	500	200		500	18		220	8
10	600	50	50	50	100	100	100	500	200	500	500	18	7.5	220	10
12	600	50	50	50	100	100	100	500	200		500	18		400	12
13 QC	600	50	50	50	100	100	100	500	200	500	500	18	7.5	700	13
15	600	50	50	50	100	100	100	500	200		500	18	7.5	700	15
18	600	50	50	50	100	100	100	500	200	500	500	18	7.5	700	18
20	600	50	50	50	100	100	100	500	200		500	18	7.5	700	20
22	600	50	50	50	200	100	100	500	200		500	18		700	22
25		50	50	50	200	100	100	500	200		500	18	7.5		25
30 QC		50	50	50	200	100	100	500	200	500	500	18	7.5		30
35		50	50	50	200	100	100	500	200		500	18			35
37		50	50	50	200	100	100	500	200		500	18			37

Note: At the depths marked QC (Quality Contol), prepare a duplicate sample for the NUT and DOC samples.

Lake Hoare Sample Volumes (ml)

DEPTH	PPR	DIC	pH	DO	CHL	NUT	DOC	CN	AN/CAT	PHYTO	PP	BAC	TDR	µl ^{14}C	DEPTH
4.5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	200	4.5
5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	200	5
6	600	50	50	50	100	100	100	500	200		500	18		200	6
8	600	50	50	50	100	100	100	500	200	500	500	18	7.5	200	8
10	600	50	50	50	100	100	100	500	200		500	18		200	10
12	600	50	50	50	100	100	100	500	200	500	500	18	7.5	310	12
14 QC	600	50	50	50	100	100	100	500	200		500	18	7.5	310	14
16	600	50	50	50	100	100	100	500	200	500	500	18	7.5	430	16
18	600	50	50	50	100	100	100	500	200		500	18		430	18
20 QC	600	50	50	50	100	100	100	500	200	500	500	18	7.5	430	20
22	600	50	50	50	100	100	100	500	200		500	18		430	22
25		50	50	50	100	100	100	500	200	500	500	18	7.5		25
30		50	50	50	100	100	100	500	200		500	18	7.5		30

Lake Fryxell Sample Volumes (ml)

DEPTH	PPR	DIC	pH	DO	CHL	NUT	DOC	CN	AN/CAT	PHYTO	PP	BAC	TDR	µl ^{14}C	DEPTH
4.5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	230	4.5
5	600	50	50	50	100	100	100	500	200	500	500	18	7.5	230	5
6	600	50	50	50	100	100	100	500	200	500	500	18	7.5	230	6
7	600	50	50	50	100	100	100	500	200		500	18		230	7
8	600	50	50	50	100	100	100	500	200	500	500	18	7.5	470	8
9 QC	600	50	50	50	100	100	100	500	200	500	500	18	7.5	470	9
10	600	50	50	50	100	100	100	500	200	500	500	18	7.5	500	10
11	600	50	50	50	100	100	100	500	200		500	18	7.5	500	11
12	600	50	50	50	100	100	100	500	200	500	500	18		500	12
15 QC		50	50	50	100	100	100	500	200	500	500	18	7.5		15
18		50	50	50	100	100	100	500	200		500	18	7.5		18

Note: At the depths marked QC (Quality Control), prepare a duplicate sample for the NUT and DOC samples.

Lake Miers Sample Volumes (ml)

DEPTH	PPR	DIC	pH	DO	CHL	NUT	DOC	CN	AN/CAT	PHYTO	PP	BAC	TDR	µl 14C	DEPTH
5	600	50	50	50	200	100	100	500	200	500	500	18	7.5	100	5
7	600	50	50	50	200	100	100	500	200	500	500	18	7.5	100	7
9 QC	600	50	50	50	200	100	100	500	200	500	500	18	7.5	100	9
11	600	50	50	50	200	100	100	500	200	500	500	18	7.5	100	11
13	600	50	50	50	200	100	100	500	200	500	500	18	7.5	100	13
15	600	50	50	50	200	100	100	500	200	500	500	18	7.5	200	15
16	600	50	50	50	200	100	100	500	200	500	500	18	7.5	200	16
17	600	50	50	50	200	100	100	500	200	500	500	18	7.5	200	17
18 QC	600	50	50	50	200	100	100	500	200	500	500	18	7.5	200	18
19	600	50	50	50	200	100	100	500	200	500	500	18	7.5	200	19

Note: At the depths marked QC (Quality Control), prepare a duplicate sample for the NUT and DOC samples.

BOTTLE WASHING

To make 1% HCL, add 90 ml (0.09L) concentrated HCL to 9L DIW.

Sample Bottles
1L amber wide mouth bottles
Dump 1% HCL that was in bottles over winter. Fill bottles with 500 ml DIW. Replace cap. Let sit right-side up for 1 hour; let sit upside down for 1 hour. Rinse 3X with DIW.

DOC
125 ml amber borosilicate glass bottles
Dump samples from previous year from bottles and remove tape, rinse 3X with hot tap water, rinse 1X with DIW, fill half-way with 1% HCL and cap with new Teflon lined cap, let sit right-side up for at least 1 hour, flip upside down for at least 1 hour, rinse with DIW 3X. Remove caps and place foil loosely over top of bottle. Combust at 475° C for 4 h. Replace caps once bottles are cooled. Tape bottles with any color tape.

Include extra bottles for QC.

Anion/Cation
125ml clear wide mouth Nalgene bottles.

Anions: Wash with DI water by rinsing 3 times, then tape with blue label tape.

Cations: Acid rinse with 1% HCL for up to 1 hour: Fill half-way with 1% HCL, cap, let sit right-side up for ~1 hour, flip upside-down for ~1 hour, DIW rinse 5 times, and then tape with white labeling tape. 1% HCL can be re-used for other acid rinsing since the bottles are new.

Include extra bottle for blank.

Nutrients
125 ml clear narrow mouth HDPE bottles
Acid wash w/ 1% HCL: Fill half-way with 1% HCL, cap, let sit right-side up for ~1 hour, flip upside-down for ~1 hour, DIW rinse 5 times. Tape with white labeling tape. 1% HCL can be re-used for other acid rinsing since the bottles are new.

Include extra bottles for QC.

DIC
30 ml serum vials
Bottles from the previous year can be re-used: check for salt build-up before using. If they are clean, rinse with 1% HCL, then rinse 6X with DIW. It is best to clean bottles at the end of the season to avoid salt build-up for the next year. If bottles are brand new, they can be DIW rinsed 6X before use.

FILTER PREP

CHL filters

25-mm GF/F filters, pre-combusted and acidified.

Combustion protocol: combust at 475^{o}C for 4 hrs spread out on aluminum foil that will serve as a wrapper after combustion and acidification.

Acidification protocol: soak in 1% HCL for 3-4 hours, rinse 4X with DIW, bring the pH to 8-9 with NaOH, rinse 2X with DIW, lay out on combusted foil to dry, and wrap in foil once dried.

Combusted and acidified filters are only necessary if certain filtrate samples are being collected – they are not required for Chlorophyll analysis. Since combusted filters are required for Dissolved Organic Carbon (DOC) filtrate (collected from one Chlorophyll replicate), and acidified filters should be used for nutrient (Soluble Reactive Phosphorus) filtrate (collected from one Chlorophyll replicate), it is recommended to combust and acidify chlorophyll-a filters.

CN filters

25 mm GF/F filters, combusted

Combust at 475° C for 4 h spread out on aluminum foil that will serve as a wrapper after combustion.

PP filters

25-mm GF/F filters, pre-combusted (to remove organic phosphorus) and acidified. Combustion protocol: combust at 475^{o}C for 4 hrs spread out on aluminum foil that will serve as a wrapper after combustion and acidification.

Acidification protocol: soak in 1% HCL for 3-4 hours, rinse 4X with DIW, lay out on combusted foil to dry, and wrap in foil once dried.

Filtration Notes

Remember: Always rinse filter towers, graduated cylinders, and filter forceps between samples to reduce cross-contamination.

Remember: Always check vacuum on vacuum pump. 6-7 inches Hg vacuum is max for CHL and PPR. 10 is max for PP, CN and AN/CAT.

Chlorophyll-a, Nutrient, and Dissolved Organic Carbon Analyses

- Use bell jars with bottle stands, 25 mm polysulfone filter towers, combusted GF/F filters.
- Take sample aliquots from 1000 ml amber bottles.
- Decant 100 ml of sample (CHL, NUT, DOC) into a graduated cylinder (don't forget to invert sample).
- Darken the room as much as possible. Filter 100 ml of sample for NUT into an acid washed 125 ml narrow mouth bottle. After the samples finish filtering, turn off the vacuum pump and wait a few seconds for the vacuum to clear. Remove the GF/F filter, fold in half (organic matter inside) and place in a labeled glassine envelope. Next, for DOC, filter 100 ml of sample into a combusted 100 ml amber glass bottle. Again, filter is folded and placed in the glassine envelope. Use one envelope for both filters. Both filters will be used for replicate Chl-a analysis.
- Sample Preservation:
  CHL: Wrap all of the glassine envelopes in foil, label the pack, and freeze.
  NUT: Place samples in a freezer (if possible) or the coldest place possible.
  DOC: Add 1 ml of 6N HCL, store bottles in their boxes at 4°C, DO NOT FREEZE

Particulate Carbon and Nitrogen

- Use a six place manifold with 25 mm polysulfone filter towers and combusted GF/F filters.
- Take sample aliquot from 1000 ml amber bottle.
- Decant 500 ml of sample into a 500 ml graduated cylinder and filter the entire volume. **Note**: The entire volume should be filtered, even for chemocline depths at Lake Bonney and Lake Fryxell. Record the volume of water filtered for each depth in each lake.
- After filtering, rinse filter while still in filter tower with approximately 20 ml of *DI* to remove salts. Remove filter with forceps, place in a labeled aluminum pan and let the filter dry. When dry, stack pans, wrap with a piece of labeling tape, put stack in a zip lock and freeze.

Anions/Cations

- Take 100 ml sample aliquot from 1000 ml amber bottle.

- Use bell jars with bottle stands, 47 mm magnetic filter towers and 0.4 μm nuclepore filters. Be careful to place the filter correct side up (see package instructions) and only handle the filter at the edge with forceps. The filters can be torn easily. When in doubt, throw it out!

- Pour sample from amber bottle directly into *DI* rinsed filter tower, no need to measure sample, just use the markings on the filter towers as a guide. First filter 100 ml of sample into an acid rinsed (white tape) 125 ml wide-mouth bottle for the cation sample. When finished, replace the acid washed bottle with a *DI* rinsed (blue tape) 125 ml wide-mouth bottle for the anion sample. Use the same filter tower for both the anion and cation aliquots. Use a new filter between the cation and anion samples (of the same depth) if filtering is slow; otherwise, you can use the same filter for both. Also filter at least one filter blank using same procedure. Pack the AN and CAT samples in separate plastic bags and place in coolers. OK to freeze.

Preparation of Standard Solutions

$$\begin{pmatrix}\text{vol. of final}\\ \text{soln. in ml}\end{pmatrix}\times\begin{pmatrix}\text{conc. of final}\\ \text{soln. in mg/ml}\end{pmatrix}=\begin{pmatrix}\text{wt. of salt}\\ \text{req. in mg}\end{pmatrix}\times\left(\frac{\text{At. wt. of element}}{\text{Formula wt. of salt}}\right)$$

Example: Prepare 500 ml of a stock molybdenum solution containing 0.1 mg (100 micrograms) of molybdenum per ml using $Na_2MoO_4 \cdot 2H_2O$.

At. Wt. of Mo = 95.95

Formula Wt. of $Na_2MoO_4 \cdot 2H_2O$ = 241.98

(500) × (0.1) = Wt. of salt required × (95.95/241.98)

Wt. of salt required = (241.98/95.95) × (500) × (0.1)

Wt. of salt required = 126.1 mg

Therefore place 126.1 mg of hydrated sodium molybdate in a 500 ml volumetric flask and add *DI* water up to the mark. Vortex well.

Dilutions
Sometimes it is necessary to prepare a more concentrated stock solution and then make sub-dilutions from it. This may be necessary when the amount of salt required is too small to be accurately weighed or for the solutions which do not store well when very dilute.

$$\begin{pmatrix}\text{vol. of final}\\ \text{soln. in ml}\end{pmatrix}\times\begin{pmatrix}\text{conc. of final}\\ \text{soln. in mg/ml}\end{pmatrix}=\begin{pmatrix}\text{vol. of stock soln.}\\ \text{required in ml}\end{pmatrix}\times\begin{pmatrix}\text{conc. of stock}\\ \text{soln. in mg/ml}\end{pmatrix}$$

Example: Prepare 100 ml of molybdenum solution containing 0.005 mg (5 micrograms) of molybdenum per ml using the previously prepared stock solution.

(100) × (0.005) = Vol. of stock soln. × (0.1)

Vol. of stock soln. = (100 × 0.005)/0.1 = 5 ml

therefore 5 ml of the stock solution should be added to a 100 ml volumetric flask and enough deionized water added to bring the volume up to 100 ml.

Molar Solutions
A one molar (1 M) solution contains one gram molecular weight of a salt in enough water to make one liter.

$$\text{Wt. of salt req. in mg} = \begin{pmatrix}\text{Vol. of final}\\ \text{soln. in ml}\end{pmatrix} \times \begin{pmatrix}\text{Molarity of}\\ \text{final soln.}\end{pmatrix} \times \begin{pmatrix}\text{Gram molecular}\\ \text{wt. of the salt}\end{pmatrix}$$

Example: Prepare 250 ml of 0.4 M sodium molybdate.

Wt. of salt required = 250 × 0.4 × 241.98
Wt of salt required = 24,198 mg = 24.198 grams.

Therefore, dissolve 24.198 grams of the hydrated sodium molybdate in enough water to make 250 ml.

Dilutions

$$\begin{pmatrix}\text{Vol. of final}\\ \text{soln. in ml}\end{pmatrix} \times \begin{pmatrix}\text{Molarity of}\\ \text{final solution}\end{pmatrix} = \begin{pmatrix}\text{Vol. of stock}\\ \text{soln. req. in ml}\end{pmatrix} \times \begin{pmatrix}\text{Molarity of}\\ \text{stock soln.}\end{pmatrix}$$

Example: Prepare 100 ml of 0.87 M sodium molybdate from a 3 M stock solution.
(100) × (0.87) = Vol. of stock required × 3.0
Vol. of stock required = (100 × 0.87)/3.0 = 29 ml
Therefore, 29 ml of the 3.0 M stock solution are diluted up to 100 ml with *DI* water.

Normal Solutions
A one normal (1N) solution contains one gram equivalent weight of a salt in enough water to make one liter. The equivalent weight of an acid is the weight which yields one mole of hydrogen ions. The equivalent weight of a base is the weight which reacts with one mole of hydrogen ions. The molar formulas can be used by substituting gram equivalent weights for gram molecular weights and normalities for molarities.

Miscellaneous
1 microgram atom of a particular element equals the atomic weight of that element expressed in micrograms (i.e. A solution containing one microgram atom of molybdenum per liter would contain 95.95 micrograms of molybdenum per liter).

0.001 grams = 1 mg = 1000 micrograms

for dilute solutions;
1 ppm = 1 mg/liter
1 ppb = 1 microgram/liter = 1 mg/m^3
0.05% of a constituent equals 500 ppm because 0.05% = 0.05/100 = $500/10^6$ = 500 ppm

Preparation of Common Acid Solutions

1. Prepare the following reagents by carefully adding the required volume of concentrated acid to the desired volume of DIW (stir continuously). **Never** add water to concentrated acid.

Table 13. Dilution factors for common acid solutions.

Desired Solution	Hydrochloric acid (HCl)	Sulfuric acid (H_2SO_4)	Nitric acid (HNO_3)
Percent active ingredient in concentrated reagent	36-37	96-98	69-70
Normality of concentrated reagent	11-12	36	15-16
Volume (ml) of concentrated reagent to prepare 1 l of:			
18 N solution	-	500 (1 + 1)	-
6 N solution	500 (1 + 1)	167 (1 + 5)	380
1 N solution		28	64
0.1 N solution	8.3	2.8	6.4
Volume (ml) of 6 N reagent to prepare 1 l of 0.1 N solution	17	17	17
Volume (ml) of 1 N reagent to prepare 1 l of 0.02 N solution	20	20	20

Spectrophotometry

It is often possible to determine the concentration of a constituent in water by reacting it with chemicals to form a colored species, and then measuring the light absorbance of this species. For this method to work, the constituent to be measured must be the limiting reactant in the fraction forming the colored species. All other reactants are added in excess. For example, dissolved oxygen concentrations may be measured by determining the amount of light absorbed by the yellow iodine solution produced in the Winkler Method. This is possible since the amount of yellow iodine released is limited by the amount of oxygen present.

To understand spectrophotometry you need to understand factors affecting light transmittance. The amount of light transmitted through a medium decreases in a geometric progression as the length of the optical path increases arithmetically. Also, for a given optical path, the transmittance decreases in a geometric progression. These relations can be expressed in the Fundamental Law of Spectrophotometry, The Beer-Lambert or simply Beer's Law:

$$A = \log\frac{1}{T} = abc$$

where

$$T = \text{transmittance} = \frac{\text{light intensity}}{\text{incident light intensity}}$$

a = absorptivity of compound
b = optical path length
c = concentration of compound
A = absorbance

Absorbance is an easy term to use in spectrophotometry since it is normally related linearly to concentration and path length. Beer's law is only strictly applicable for a single wavelength of light. In practice, a narrow band width of light is produced by a colored filter or by a monochromator. This wavelength selected for a particular analysis is usually that which produced the greatest difference in absorbance between the colored species of interest and any interfering compound (i.e. , color introduced in reagents, turbidity).

Photometers are most accurate when readings on samples fall in the range of 0.1 to 1 absorbance with respect to a blank adjusted to read 0 absorbance (100% transmittance). The analyst can adjust the path length of light by selecting the proper spectrophotometer curvette. Path lengths of 1,4,5, & 10 cm are commonly used. By selecting the proper path length, absorbance can often be made to fall in the proper range.

In its simplest form, the concentration of an unknown substance can be determined by: (1) Adding reagents which will react with the substance to form a colored species; (2) Measuring the absorbance of the colored complex at an appropriate wavelength; (3) Constructing a standard curve by measuring the absorbances of known concentrations of the substance after treatment with the color forming reagents. If Beer's law is followed, a linear relationship between

concentration and absorption is found and the line will pass through the origin; (4) Comparing the absorbance of the unknown against the standard curve to estimate its concentration.

There are three factors which often interfere with this simple procedure. (1) Turbidity or color of the sample water may cause additional absorbance of light in addition to that of the colored species of interest. (2) The reagents themselves often introduce significant quantities of the constituent of interest. (3) The distilled water used for the standard solutions and reagents may contain significant quantities of the constituent to be measured.

I. If #1 and #2 are important the following procedures should be followed:

a. The standard curve is prepared by balancing the photometer to zero absorbance with plain distilled water and reading all the standards, including a zero standard (Reagent blank) against the distilled water. If significant contamination is present in the reagents, the line relating concentration to absorbance will not pass through the origin.

b. To correct for #1, the sample to be tested is divided into two aliquots. One is treated with all of the reagents except the color-forming reagent. This sample blank is then placed in the photometer and it is adjusted to read zero absorbance by adjusting the slit width.

The aliquot with the color-graming reagent is then tested, and its absorbance is compared with the standard curve and concentrations calculated directly. Any significant increase or decrease is volume caused by the omission or addition of reagents must be considered in the calculations.

II. In some situations, the distilled water used to prepare the reagents and the standards may have significant quantities of the constituent to be measured. If it is known that this is the case, and the reagents contribute insignificant contamination, a special procedure is necessary. After treatment, all of the reagent blanks, standards, and water samples contain the same amount of reagents, but different amounts of distilled water. The standards all contain 100% distilled water. Therefore, in constructing the standard curve the full value of the reagent blank (0 standard) is subtracted from each standard.

$$(A_{corr} = A_{standard} - A_{reagent\ blank})$$

A_{corr} is then plotted against concentration. That is, the standard curve is adjusted to pass through the origin.

To measure the sample absorbance, the photometer is first adjusted to zero absorbance using the sample blank as described previously. The absorbance of a sample with the color-forming reagent present must be corrected for the amount of constituent added by the distilled water. For example, assume that we are adding 5 ml of reagents to 100 ml of lake water. The true absorbance of the samples is calculated as follows:

$$A_{corr} = A_{sample} - \frac{5}{105} A_{reagent\ blank}$$

Once a standard curve has been constructed and if it is found to be a linear relationship, it is often easier to use a linear regression equation to determine concentrations than to manually plot absorbance values on graph paper.

For example, in I above, a relationship between iron concentration (ppb) in the standards and absorbance might be:

$$\text{Absorbance} = .00343 \times (\text{conc.}) + 0.0333 \text{ (as in Figure 1)}$$

If the absorbance of the lake water sample is known, its concentration can then be easily calculated.

Once the relationship between concentration and absorbance is known, a complete set of standards need not be prepared for every single set of samples to be analyzed. However, it is necessary to prepare a reagent blank and at least one standard in the upper end of the optimum concentration range, along with every group of samples, in order to verify any unsuspected changes in the reagents, instruments, or the technique. At regular intervals a complete set of standards (5-6) should be prepared. The standards should cover the range of values expected from the water samples.

It is possible that a water sample will contain solutes that will interfere with detection (i.e. color formation) of the species of interest. To determine if this is occurring, an internal standard can be run. In this procedure a lake water sample is split into two series. A known quantity of the species if interest is added to each flask in the first series. For example, 10 $\mu g \cdot$ atoms Fe-liter^{-1} might be added to each container. Flasks in the second series would receive an equal amount of distilled water. The two samples are then normally processed. If the mean of the second series was 5.0 $\mu g \cdot$ atoms $\cdot$ fe-liter^{-1}, the mean of the first series with the internal standards should yield a value of 15 $\mu g \cdot$ atoms Fe $\cdot$ liter^{-1}. If the second value differed significantly from 15.0, interference is probable. Internal standards may be used in other types of quantitative analysis besides calorimetric analysis.

References

APHA. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. A.E. Greenberg, Clesceri, L.S., and A.D. Eaton eds.. APHA, Washington, DC.

Preparation of ^{14}C-bicarbonate Stock Solution

$$\frac{75\,\text{mCi}}{75\,\text{ml}} \times \frac{1000\,\mu\text{Ci}}{1\,\text{mCi}} = \frac{75{,}000\,\mu\text{Ci}}{50\,\text{ml}}$$

$$\frac{75{,}000\,\mu\text{Ci}}{\text{X ml}} = \frac{100\,\mu\text{Ci}}{1\,\text{ml}}$$

$$\text{X ml} = \frac{75{,}000\,\mu\text{Ci}}{100\,\mu\text{Ci}}$$

$$\text{X ml} = 750\,\text{ml}$$

$$\therefore \text{Add}\,\frac{75\,\text{mCi}}{75\,\text{ml}}\,\text{to}\,675\,\text{ml DIW}$$

1. Prepare the ampoules for ampulation. Autoclave the ampoules if they appear unclean.

Quantity of ampules required (one scenario)	
Qty × ampoule vol.	Total Volume (ml)
28 × 15 ml	420
28 × 10 ml	280
6 × 5 ml	30
10 × 2 ml	20

2. Boil 675 ml of nanopure/organopure water and cool. Place a stir bar in the flask and stopper the flask with an ascarite scrubber. This will prevent CO_2 from entering the flask during the cooling period. **Note:** There is a special flask for this procedure in the ampulating kit, Crary Laboratory.
3. Adjust the pH of the water to 10 with 5N NaOH (final pH should be 9.5-10.0).
4. Add the 75 mCi of ^{14}C-bicarbonate stock to the 675 ml of boiled nanopure water.
5. Recheck the pH and adjust accordingly.
6. Ampulate while mixing the stock solution.
7. Autoclave the ampoules (slow-cool setting) in phenolpthaline bath to detect leaks (leaky vials will suck in liquid upon cooling, and the phenolphthalein will turn the ampoule pink by reacting with the pH 9.5 ^{14}C-bicarbonate solution).

Phenolthalein indicator solution: dissolve 5 g phenolthalein disodium salt in distilled water and dilute to 1 l.

8. Wash the ampoules thoroughly, and record the total number and volume of ampoules prepared.

Preparation of ^{14}C-toluene Quench Curve and Standardization

1. Use a certified ^{14}C-toluene standard and calibrated P100 Gilson Pipetman to prepare quench curve standards.
2. Calculate the present day activity (N_t) of the ^{14}C-toluene standard.

   $$N_t = N \times e^{(k \times t)}$$

   where N is the specific activity at date of manufacture, k is the decay constant for ^{14}C (-0.0001210968), and t is the elapsed time in years since manufacturing.

   Note: 2.2×10^6 dpm μCi^{-1}

3. Prepare each quench standard according to the following table, the volume of ^{14}C-toluene will represent a known dpm added to each vial. Count each vial and determine the actual dpm for each vial before adding the acetone.

Vial #	Volume of ^{14}C-toluene added (µl)	Volume of Acetone added (µl)
Q1	25	0
Q2	25	50
Q3	25	100
Q4	25	200
Q5	25	300
Q6	25	400
Q7	25	500
Q8	25	800
Q9	25	1000
BLANK	0	0

4. Add 10 ml of cytoscint cocktail to each 20 ml scintillation vial and recount the Quench Curve.
5. Compare the new Quench Curve to the old one and determine the percent difference:

$$\% \text{ difference} = \left[\frac{\left(\text{Average old dpm vial}^{-1} - \text{Average new dpm vial}^{-1} \right)}{\text{Average old dpm vial}^{-1}} \right] \times 100$$

Preparation of ^{3}H-toluene Quench Curve and Standardization

1. Use a certified ^{3}H-toluene standard and calibrated P100 Gilson Pipetman to prepare quench curve standards.
2. Calculate the present day activity (N_t) of the ^{14}C-toluene standard.
   $N_t = N \times e^{(k \times t)}$
   where N is the specific activity at date of manufacture, k is the decay constant for ^{3}H (-0.0565373), and t is the elapsed time in years since manufacturing.

   Note: 2.2×10^{6} dpm μCi^{-1}

3. Prepare each quench standard according to the following table, the volume of ^{14}C-toluene will represent a known dpm added to each vial. Count each vial and determine the actual dpm for each vial before adding the acetone.

Vial #	Volume of ^{3}H-toluene added (µl)	Volume of Acetone added (µl)
Q1	25	0
Q2	25	50
Q3	25	100
Q4	25	200
Q5	25	300
Q6	25	400
Q7	25	500
Q8	25	800
Q9	25	1000
BLANK	0	0

4. Add 20 ml of cytoscint cocktail to each 20 ml scintillation vial and recount the Quench Curve.
6. Compare the new Quench Curve to the old one and determine the percent difference:

$$\% \text{ difference} = \left[\frac{\left(\text{Average old dpm vial}^{-1} - \text{Average new dpm vial}^{-1} \right)}{\text{Average old dpm vial}^{-1}} \right] \times 100$$

Nikon Labophot Microscope Notes

The Nikon Labophot located in the Priscu lab is ** with an HBO 100W/L2 mercury lamp. The mercury bulb should be changed every 200 hours according to manufacturer instructions. The Priscu lab follows a schedule of changing the bulb every 500 hours per instruction by John Priscu. The schedule of bulb changes since 1996 is as follows:

Date	Hours Logged on previous Bulb	Notes
1 April 1996		New bulb
14 March 1998	210	New bulb installed
29 January 2003	525	New bulb installed; Hour logger reset
6 October 2008	1162	New bulb installed; Hour logger reset

Instruction for changing the Mercury Lamp Bulb

To take the lamp socket out of the scope – remove 2 small black screws on 10 and 2 positions on top of the lamp socket (sticks out of right side of lamp housing, which is the big black box on the back of the scope).

To take the bulb out of the lamp socket – loosen rigid (base) end screw first, raise bulb out, then loosen loose end screw and take bulb out.

If you accidently touch the bulb – clean it off with alcohol and lens paper or the oils from your hand will burn into the bulb.

To re-install new bulb into lamp socket
The base and top of the lamp socket are 2 different diameters, so you can't put the bulb in backwards. Make sure the bulb seats all the way in so the silver part at the end of the bulb disappears. Hold the bulb at the top and tighten screw (don't hold actual bulb, hold the covering on the end). Make sure the bottom screw is loose, push bulb in, and tighten. Only the glass part of bulb should be showing.

Instruction for centering the Mercury Lamp Bulb
Put a white piece of paper on the stage and take out one of the objectives.

The 3 screws on the back of the lamp housing are for moving mirrors in inside to center bulb – you shouldn't need to use these when a new bulb is installed.

1). The two screws on the right side of the lamp housing (vertical and horizontal) are primary adjustments (1 on top and 1 on side). Bring 2 light points showing on stage so that 1 is above and 1 is below each other.

2). Collector focusing knob (most forward knob on right side of housing) – adjusts for even illumination. Put slide in and look through objective (20X works). Move knob until get even illumination – fill field with light by turning knob.

3). Centering tool – The centering tool screws into the place of an objective. Use vertical and horizontal (primary adjustment) knobs to move 2 bright light spots in centering tool (can see which one is mirror image by moving mirror adjustments on back and seeing which one moves). You want 2 light points sitting edge to edge vertically in the middle of the centering tool cross-hairs.

4). Go back to slide on 20X objective – check collector knob illumination.

Note:
Don't put immersion oil next to the mercury lamp power supply because it will heat up the oil and change the refractive index.

Contact for Nikon Labophot:

Jan A. Strelow
Vice President
Meridian Instrument Co., Inc.
P.O. Box 519
Freeland WA. 98249
Answering service 360 331-0266
Fax 360 331-1297
Cell 206 940-9752

Jan Strelow supplies the mercury bulbs, but they can also be ordered:

Lamp Technologies
1654 Sycamore Ave
Bohemia NY 11716
1-800-533-7548

Key:
element name
atomic number
element symbol
1997 atomic weight (mean relative mass)
*lanthanoids
**actinoids

Lake Ice Thickness and Piezometric Lake Depth

(method used until 0304 season – this parameter continues to be measured, but a new method has been implemented)

General Discussion

Lake ice thickness measurements are recorded at each hole drilled in the ice cap; this includes all sampling holes, the incubation hole, sediment trap holes and blue box holes. However, in an effort to better monitor annual lake ice variation and to randomize measurements, a 1000 m^2 (100 × 100 m) sampling grid has been created on each lake (Lakes Bonney; east and west lobe, Hoare, and Fryxell). Each grid is located along a 100 m baseline due south of the blue instrument box on each lake (See Section 2.1). Within this grid, 10 random ice thickness measurements are performed during the third and fourth weeks of November.

Materials

100 m tape measure (2)
2" cutting bit extensions (2)
2" flight extensions (8)
Badger power head
Ice thickness measuring tape: A 10 m tape measure attached to the mid-point of a 30 cm long brass rod, with 10 m of $^1/_8$" rope attached to one end of the rod.
Messenger

Procedure

Before drilling the 10 random ice holes in the 1000 m^2 grid, generate ten pairs of random numbers between 0 and 100 and use these as south and east coordinates (west coordinates for Lake Hoare) for each lake. For example, if the sampling coordinates are (56S, 72E), the hole would be located 56 m due south along a baseline from the blue instrument box, then similarly, 72 m due east of that baseline. At Lake Hoare, the easterly coordinates are changed to west due to extreme ice conditions not representative of the entire ice cap.

To begin sampling, stretch out a 100 m tape due south of the blue instrument box and secure both ends. Similarly measure the easterly distance from the baseline and mark the coordinate with a flagged ice screw. After all of the sample locations have been identified, begin drilling a 2" ice hole at the first location. Once the hole completely penetrates the ice cap, take 2 flight extensions (attached to one another) and use a plunger to remove as much slush as possible **Note: Do Not** drop the extensions otherwise they will be lost forever! Lower the brass rod completely through the ice layer and pull upward on the tape measure causing the rod to catch the bottom of the ice. Record the distances between the bottom of the ice cover and the piezometric water level (z-water) and the top of the ice cover (z-ice). The piezometric water level is the level at which water rises up through the ice hole due to hydrostatic pressure caused by the permanent ice cover. Viewing the piezometric water level may be difficult when it is 30 cm or more below the top of the ice surface. Therefore, move the tape measure in a side to side motion, pushing as much slush to the sides of the hole then record the distance to the water level. Subtract z-water from z-ice to obtain freeboard (z-difference). Together these 3 measurements provide some

insight into the topographical features of the ice cover as well as the density of the ice. **Note:** Correct for offset due to the way the brass rod is attached to the tape measure.

Piezometric lake depth is measured at each sampling hole the day before a limno run is performed. Attach a sample messenger to the end of a 100 m tape measure and lower the messenger to the lake bottom. Record the piezometric water level in the sampling hole. **Note**: Adjust the depth measurement to account for offset due to messenger.

Protozooplankton Enumeration and Biomass
(method used until ?? season)

General Discussion

Light microscopy is used to count and identify ciliates and epifluorescence microscopy is used to enumerate flagellates. A number of methods exist to measure ingestion rates of protozoa including the dilution method (Landry, 1993), genetically marked minicells (Wikner, 1993) and the use of fluorescently labeled prey (Sherr and Sherr, 1993). The latter method was chosen, as grazing rates can be determined over a short time period, there is minimum manipulation of the sample, the protozoan predator species can be identified and prey selection can be assessed. When possible fluorescently labeled algae (FLA) and fluorescently labeled bacteria were used in preference to fluorescently labeled microspheres (FLM) as certain protists have been found to actively select against FLM (Nygaard *et al*., 1988). Growth was measured directly by measuring abundance changes in a water sample (McManus, 1993). Advantages of this method include the additional information that can be gained (such as cell size), that growth is measured directly and not by some biochemical indicator (e.g. carbon and ATP) and that it is not technically difficult to perform. This method is labor intensive, however, and changes in species composition can take place over the relatively long incubation periods.

Materials

1 μm and 2 μm 25 mm polycarbonate membrane filters
500 ml graduated cylinders
5 μm 25 mm backing filters
Centrifuge
Epifluorescence microscope with ultraviolet and blue filters.
Filter rack with waste trap and vacuum pump
Glass filter set 25mm x 15ml
Glass slides and cover slips
Immersion oil (ultra low fluorescence grade)
Line graticule
Sedgewick Rafter counting chamber
Whipple grid

Reagents

Phosphate buffered glutaraldehyde
DAPI: 4', 6-diamidino-2phenylindole. Dissolve 10mg of DAPI in 100ml of distilled water.
Lugol's solution: Dissolve 20 g potassium iodide (KI) and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid.

Procedure

Nanoflagellates (modified method of Booth, 1993)

1. Decant 54 ml of sample from a 1000 ml HDPE clear bottle into a 60 ml HDPE widemouth bottle, prepare duplicates.

2. Preserve samples with 6 ml of phosphate buffered glutaraldehyde (2% final concentration) and store in the dark at 4°C before analysis. Analyze the samples within one month to avoid fading of the autofluorescence.

3. Place a 5 μm backing filter onto the glass filter base followed by a 1 or 2 μm polycarbonate membrane filter and replace tower. The 5 μm backing filter will help minimize nonrandom distribution.

4. Decant 50 ml of the sample into the glass filter funnel apparatus and stain with three drops of DAPI. Filter sample under a low vacuum (<7 in Hg).

5. Remove the polycarbonate filter and mount onto a glass slide. Add a drop of low-fluorescence immersion oil in the middle of the filter and place the cover slip on top.

6. Examine the slide immediately under epifluorescence at a magnification between 400× and 1000×.

7. Count all the flagellates under the UV filter and then switch to the blue filter in order to distinguish phototrophic nanoflagellates (PNAN) from heterotrophic nanoflagellates (HNAN). Under the blue filter, phototrophic organisms will fluoresce red, whereas the heterotrophic organisms will show no red fluorescence. Count the number of HNAN and PNAN in 20 Whipple grids. If the abundance is low continue to count until at least 100 PNAN and 50 HNAN have been counted.

8. Measure the effective filter funnel diameter.

    4. Calculate cell abundance from:

$$\text{cells ml}^{-1} = \frac{(\#\text{ of cells counted} \times \text{funnel area})}{(\text{area of count} \times \text{dilution factor} \times \text{vol filtered})}$$

Dilution factor is dependent on the volume of glutaraldehyde added and is calculated:

$$\text{dilution factor} = \frac{\text{volume of water sample}}{\text{total volume of sample} + \text{glutaraldehyde}}$$

11. To estimate biovolume, select the nearest geometric shape that approximates the organism, and measure the length and width of the cells at 1600x using a line graticule.

12. To convert biovolume to biomass, use a carbon conversion factor of 200 fg μm^{-3} (Borsheim and Bratbeck, 1987).

Ciliates

1. Decant 500 ml of sample directly from the Niskin water sampler into a 500 ml HDPE amber bottle.

2. Preserve samples to a final concentration of 1% Lugol's solution.

3. Settle the samples in 500ml graduated cylinders for 5 days.

4. Siphon off surface water until approximately 50 ml of the sample remains. Stop siphoning if the bottom is disturbed.

5. Place sample into a 50 ml centrifuge tube and centrifuge at 1000 rpm for 12 minutes.

6. Concentrate the sample to 1ml and store in the refrigerator before analysis.

7. Count and identify ciliates in a Sedgewick-Rafter counting chamber at x160.

8. Determine ciliate sizes using a line graticule at 320x and calculate biovolume using the nearest geometric shape.

9. Convert biovolume to biomass using a carbon conversion figure of 190 g C μm^{-3} (Putt and Stoecker, 1989).

Protozooplankton Feeding and Growth Rates
(method used until ?? season)

General Discussion

When measuring growth rates of protists, run two incubations simultaneously. The first sample is unscreened and is used as a control. The second sample is screened through a 10 μm mesh for nanoflagellates, or a 100 μm mesh for ciliates, to remove any predators. In the Dry Valley lakes there are no mesozooplankton predators and many of the ciliates species are capable of squeezing through a 10μm mesh. Therefore, run only one unscreened incubation.

Materials

1 μm and 2 μm 25 mm polycarbonate membrane filters
4 l container
500 ml graduated cylinders
5 μm 25 mm backing filters
Centrifuge
Epifluorescence microscope with ultraviolet and blue filters.
Filter rack with waste trap and vacuum pump
Fluorescently labeled algae (FLA) and fluorescently labeled bacteria (FLB) made from nanoflagellates and bacteria present naturally in the lakes. See Sherr and Sherr (1993) for instructions on how to make FLA and FLB.
Glass filter set 25mm x 15ml
Glass slides and cover slips
Immersion oil (ultra low fluorescence grade)
Incubator
Line graticule
Sedgewick Rafter counting chamber
Whipple grid
Whirl-pak bags or Nalgene bottles

Procedure for Feeding rates (modified method of Sherr and Sherr, 1993)

1. Decant 50 - 400 ml of sample directly from the Niskin sampler into rinsed Whirl-pak bags or Nalgene bottles depending on the concentration of protists.

2. Place samples into an incubator that approximates *in situ* light and temperature conditions of the lake.

3. Determine *in situ* bacteria and nanoflagellate abundance using the methods in the Enumeration and Biomass section.

4. Calculate the concentration of fluorescently labeled algae (FLA) or fluorescently labeled bacteria (FLB) which will be added to the samples. Add FLB to equal approximately 10% of the total bacterioplankton for ciliate uptake and about 30% of total bacterioplankton for

flagellate uptake. For ciliate ingestion, add FLA to equal about 30% of the total nanoflagellate concentration.

5. Vortex the fluorescently labeled prey (FLP) for two 5- second bursts.

6. Add the FLP and vortex the sample gently.

7. At selected time intervals (~ how much time) remove subsamples (the volume will depend on the abundance of protozoa) and preserve in ice cold, buffered glutaraldehyde to a final concentration of 2%. Store in the dark at 4^{o}C until epifluorescence analysis.

8. Stain the subsample with DAPI and filter onto a membrane filter (pore size 2 μm for nanoflagellates, 5 – 8 μm for ciliates).

9. Examine using epifluorescence. Locate individual protistan cells using the UV filter set and then switch to the blue filter set to inspect each cell for ingestion of FLP. Count the number of FLP ingested by 50 individual protistan cells and determine the concentration of protists in the sample.

10. Plot FLP protist^{-1} against time. A linear regression model will provide a rate of FLP uptake by the protists of interest. Divide the FLP uptake rate (FLP protist^{-1} h^{-1}) by the concentration of FLP to obtain an hourly clearance rate (ml protist^{-1} h^{-1}). Multiply the clearance rate by the *in situ* prey abundance to obtain individual ingestion rates (prey protist^{-1} h^{-1}). The community grazing rate (prey ml^{-1} d^{-1}) can be calculated by multiplying the ingestion rate (ml protist^{-1} d^{-1}) by the protist concentration (protist ml^{-1}). Calculate the assemblage clearance (d^{-1}) by multiplying the clearance rate (ml protist^{-1} d^{-1}) by the protist concentration (protist ml^{-1}).

Procedure for Growth Rates (modified method of McManus, 1993)

1. Gently pour 3-4 l of sample into a 4 l HDPE bottle.

2. Incubate the sample at *in situ* light and temperature conditions.

3. At given time intervals (this will depend on the protist in question) remove at least 3 subsamples and fix with buffered glutaraldehyde, final concentration 2%. There should be at least 8 time intervals over the duration of one experiment.

4. Count protist abundance in each subsample using the methods in the Enumeration and Biomass section.

5. Assuming population dynamics respond exponentially, the following equation may be use to calculate the populations exponential growth rate:

$$k = \ln\left(\frac{N_t}{N_0}\right)\frac{1}{t}$$

where k is the intrinsic growth rate, and N_t and N_0 are the abundances at the final and initial sampling times. As multiple time points are sampled, a linear regression of ln (N_t) vs time will have a slope equal to k. However, the exponential growth model may not always be appropriate. If the growth trajectory is linear, this may indicate the population is near the inflection point of the logistic curve, in which case the growth rate may be calculated by:

$$k = \frac{dN}{dt}\left(\frac{1}{N_x}\right)$$

where N_x is the value of N at the midpoint of the incubation, and dN/dt is the slope of the N vs time regression.

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Bacterial Production Determined by ^{3}H Thymidine Incorporation

(method used until 0506 season – this parameter continues to be measured, but a new method has been implemented)

General Discussion

Despite the controversy over the shortcomings of the thymidine incorporation method, its widespread use in various systems, the relative simplicity of the method, and its repeatability make this a useful technique to determine bacterial production. We have complemented our thymidine data with leucine and adenine uptake data, and microautoradiography. Our leucine uptake method is included here for comparison. A complete review of the thymidine method may be found in Robarts and Zohary (1993) and Bell (1993). The leucine method is discussed in Kirchman, et al. (1985) and Kirchman (1993).

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

Glass scintillation vials (20 ml with HDPE cone caps)
Permanent marker
10 ml Gilson Pipetman (non-rad use)
Autoclaved 10 ml pipet tips (autoclave 20 minutes)
Acrodisc 0.2 μm filters and syringe
P20 Gilson Pipetman (rad use only)
P200 Gilson Pipetman (rad use only)
Autoclaved 200 μl pipet tips (autoclave 20 minutes)
10 ml Repipettor

MCM Crary Lab

Glass scintillation vials (20 ml with HDPE cone caps)
Permanent marker
0.2 μm, 47 mm, polycarbonate filters
Glass filter apparatus
10 ml Repipettor for cocktail in fume hood
Liquid scintillation counter

Reagents

Dry Valleys Lab

^{3}H-Thymidine (20 Ci mmol^{-1})

Trichloroacetic acid solution (10% TCA): Dissolve 100g of TCA in *DI* water, and bring volume up to 1000 ml. Store at ~1ºC. The TCA may be preweighed and placed into a 1000 ml HDPE bottle and stored until needed, then bring to volume the day of experiment.

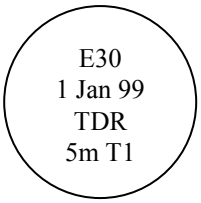
Formalin buffered with sodium borate (0.2 μm filtered)

MCM Crary Lab

Trichloroacetic acid solution (5% TCA): Dissolve 50g of TCA in *DI* water, and bring volume up to 1000 ml. Store at ~1ºC
Cytoscint or other scintillation cocktail

Procedure
Below steps to be conducted in Dry Valleys Lab

1. Label 5 scintillation vials per depth, preferably the night before sample collection. Two of the five vials are kills and should be labeled K1 and K2. Three of the vials are live treatments and should be labeled T1-T3.



2. The thymidine stock solution comes in ethanol which eliminates bacterial growth and volatile products of self-radiolysis. Therefore the ethanol must be evaporated from solution. Withdraw 200 µl of the thymidine solution and pipet into a clean 7 ml scintillation vial. The volume of thymidine solution to be dried down depends on experimental design. Radiolabeled thymidine is added to each vial to achieve a final concentration of 20 nM. If the specific activity of the ^{3}H Thymidine stock is 20 Ci mmol^{-1}, then 4 µl of stock solution must be added to each vial (See calculation of ^{3}H Thymidine addition at the end of this section). If there are 45 vials in the experimental design, then at least 180 µl of thymidine should be dried down. However, dry down 200 µl to ensure there will be enough to complete the experiment. To dry down the thymidine solution, use either filtered exhaust air from a vacuum pump or nitrogen gas and place the air flow directly over the mouth of the scintillation vial so that the air turbulence will evaporate the ethanol from solution. This procedure must be performed inside a fume hood.

3. Reconstitute the thymidine with four times as much filtered distilled water. For example, if 200 µl of thymidine solution is dried down, then add 800 µl of 0.2 µm filtered *DI* water.

4. Bacterial production samples (TDR) are taken from the 1000 ml amber HDPE bottles. Gently invert the amber bottle to thoroughly mix, withdraw 10 ml of sample and dispense into scintillation vial, replacing cap immediately. Prepare all TDR samples and preserve the Kill Treatments (K_1, K_2) by adding 0.5 ml of 0.2 µm filtered formalin (~15 drops from Acrodisc syringe filter). Thoroughly mix the kill treatments. Store these at 4ºC in the dark until you add the thymidine. (Note: you will incubate the TdR samples for 20hrs so plan your inoculation accordingly. For example it is best to kill the samples around 10am the next day so you'd want to start adding the reconstituted thymidine around 2pm. It takes ~1hr to dry down the original thymdine.)

20. Pipet 16 µl of reconstituted thymidine into each vial (20 nM thymidine final concentration) and thoroughly mix. **Note**: Start with all the live treatments, then do the kills. Note the time of the additions.
21. Perform activity checks of the thymidine by pipeting 20 µl from each of five live treatments (any depths) into scint vials.

7. Incubate the samples in the dark at 1-4ºC for 20 h. Monitor temperature, if it varies, compute a time weighted average temperature for the incubation period.

8. Prepare 10% TCA by dissolving 100g of TCA to distilled water. Bring volume up to 1000 ml. Store at ~1ºC. The TCA may be preweighed and placed into a 1000 ml HDPE bottle and stored until needed, then bring to volume the day of experiment.

9. Terminate incubation with the addition of 10 ml of ice cold 10% TCA (use repipettor on jar) to each vial. Store vials at 4ºC until filtered.

Below steps to be conducted in MCM Crary Lab

10. Label a new set of scintillation vials as described in step 1, so that once the sample is filtered the filter may be placed into a clean scintillation vial.

11. Prepare 5% TCA by dissolving 50 g of TCA into 1000 ml of distilled water. Store on ice.

12. Place 0.2 µm 47 mm polycarbonate filters onto each glass filter base and replace tower.

13. Pour sample into filter apparatus and filter under low vacuum (<7 in Hg). Rinse scintillation vial 3 times with ice cold 5% TCA and pour into filter tower. Rinse filter tower 3 times with 5% TCA. Filter until filter is dry. Place filter into its respective scintillation vial.

14. Once the samples are filtered, add 20 ml of cytoscint or comparable scintillation cocktail (use repipettor attached to cocktail bottle in hood), and count samples in a liquid scintillation counter on a calibrated ^{3}H channel. Add cocktail to activity check samples and count as well.

15. Thymidine uptake rate is determined by:

$$\text{nM TdR day}^{-1} = \left(\frac{(DPM\ treatment - DPM\ Kill)(nM\ thymidine)}{(\mu Ci)\left(\frac{2.2\text{x}10^{6}\ dpm}{1\mu Ci}\right)(t\ hr)} \right) \cdot \left(\frac{24hr}{d} \right)$$

where *DPM treatment* is the average DPM of T1-T3, *DPM kill* is the average treatment of the kills, *nM thymidine* is the final concentration of thymidine in the incubation vial (20 nM), μCi is the activity added (4 µCi), $2.2\text{x}10^{6}$ is the number of dpm μCi^{-1}, and *t* is the incubation time (h).

16. Thymidine uptake rate is corrected for the incubation temperature as follows:

$$\text{nM TdR}_t\ \text{day}^{-1} = \text{nM TdR day}^{-1} \cdot e^{\left(\frac{Ea \cdot \left[\left(\frac{1}{^{\circ}C_I + 273^{\circ}K} \right) - \left(\frac{1}{^{\circ}C_A + 273^{\circ}K} \right) \right]}{R} \right)}$$

where Ea is the energy of activation of 12,600 kcal mol^{-1}, $^{\circ}C_I$ is the incubation temperature (°C), $^{\circ}C_A$ is the ambient lake water temperature at specific depth (°C), and R is a gas constant (1.987 cal mol^{-1} $^{\circ}K^{-1}$), (Priscu, unpublished data).

17. Thymidine uptake rate is converted to bacterial production by empirically determining a carbon conversion factor for the assemblage being studied (Kirchman and Ducklow, 1993). There are also a number of carbon conversion factors reported in the literature to convert thymidine uptake rate to bacterial production. The MCM LTER uses the following conversion factors (Takacs and Priscu, 1998):

2.0×10^{18} cells mol thymidine^{-1}
10 fg Carbon cell^{-1}.

Calculation of ^{3}H Thymidine addition

Use the following equation to calculate the volume of ^{3}H Thymidine stock solution to be added to each sample to achieve a concentration of 20 nM thymidine:

$$(C_I)(V_I) = (C_F)(V_F)$$

where C_I is the initial concentration of the stock solution, V_I is the volume of stock solution added to sample, C_F is the desired final concentration of stock solution in the sample, and V_F is the total volume of the sample.

Assuming the ^{3}H stock solution has a specific activity of 20 Ci mmol^{-1} (specific activity will be noted on product), and an initial concentration of 1mCi ml^{-1}.

$$\frac{\text{mmol}}{20\,\text{Ci}}\left(\frac{\text{Ci}}{1000\text{mCi}}\right) = \frac{\text{mmol}}{20{,}000\,\text{mCi}}$$

$$\frac{\text{mCi}}{\text{ml}}\left(\frac{\text{mmol}}{20{,}000\,\text{mCi}}\right) = \frac{\text{mmol}}{20{,}000\,\text{ml}} = \frac{\text{mmol}}{20\,\text{L}}$$

$$\frac{\text{mmol}}{20\,\text{L}}\left(\frac{10^6\,\text{nmol}}{\text{mmol}}\right) = \frac{50{,}000\,\text{nmol}}{\text{L}}$$

$$\frac{50{,}000\,\text{nmol}}{\text{L}}(\text{X mls}) = \frac{20\,\text{nmol}}{\text{L}}(10\,\text{ml})$$

$$\text{X mls} = 0.004$$

$$\therefore 4\,\mu\text{l}\,(4\,\mu\text{Ci}) \text{ of stock solution should be added to each vial}$$

Because 4 µl of stock solution is not easily pipetted with great accuracy, we dilute the stock solution 4× and pipet 16 µl of dilute stock solution. Therefore, pipet 200 µl [4 µl × 45 TdR treatment vials = 180 µl + 20 µl (for insurance) = 200 µl stock ^{3}H thymidine solution] into a clean 7 ml scintillation vial and evaporate the ethanol solution. Once the ^{3}H thymidine solution is completely dry, reconstitute with 800 µl of 0.2 µm filtered *DI* water and thoroughly mix to achieve a homogeneous solution. Theoretically, 16 µl of the reconstituted solution will contain 4 µCi of ^{3}H thymidine.

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Bacterial Production Determined by ^{3}H Leucine Incorporation

(method used until ?? season – measurement of this parameter began again during the 0607 season with a new method)

General Discussion

The leucine procedure is similar to the thymidine procedure until the filtering step. Follow the above procedure, except the volume of leucine to add is different. The final concentration of leucine in each vial should be 20 nM. Our present leucine stock is 5 mCi ml^{-1}, 52 Ci mmol^{-1}, so add 10.4 µl of reconstituted stock. It is not necessary to reconstitute the leucine with 4× as much water because 10.4 µl is more easily pipetted with accuracy.

Materials

0.2 µm, 47 mm, polycarbonate filters
P10 ml Gilson Pipetman
P20 ml Gilson Pipetman
P200 ml Gilson Pipetman
Autoclaved 200 µl pipet tips
Autoclaved 10 ml pipet tips
Glass filter apparatus
Glass scintillation vials (20 ml with HDPE cone caps)
Heating block or water bath
Liquid scintillation counter
Permanent marker

Reagents

Trichloroacetic acid solution (10% TCA): Dissolve 100g of TCA in *DI* water, and bring volume up to 1000 ml. Store at ~1ºC. The TCA may be preweighed and placed into 1000 ml HDPE bottles and stored until needed, then bring to volume the day of experiment.

Trichloroacetic acid solution (5% TCA): Dissolve 50g of TCA in *DI* water, and bring volume up to 1000 ml. Store at ~1ºC

^{3}H-Leucine

Formalin buffered with sodium borate (0.2 µm filtered)

Procedure

1. Follow steps 1-8 as outlined in the thymidine uptake procedure, however, in step 5 add 10.4 µl of ^{3}H Leucine.

2. Label a new set of scintillation vials before filtration.

3. Heat samples to 80ºC for 15 minutes and cool under ambient conditions.

4. Place 0.2 µm 47 mm polycarbonate filters onto the glass filter base and replace tower.

5. Pour sample into filter apparatus and filter under low vacuum (<7 in Hg). Rinse scintillation vial 3 times with ice cold 5% TCA and pour into filter tower. Rinse filter tower 3 times with ice cold 5% TCA and follow with 3 rinses of ice cold 80% ethanol. Filter until filter is dry. Place filter into its respective scintillation vial.

6. Once the samples are filtered, add 20 ml of cytoscint or comparable scintillation cocktail, and count samples in a liquid scintillation counter on a calibrated ^{3}H channel.

Biomass production is calculated based on the following equation and literature based values.

$$\text{Production} = \text{Leu} \times 131.2 \times (\%\text{Leu})^{-1} \times (\text{C/Protein}) \times \text{ID}$$

Where Leu is the rate of leucine incorporation, 131.2 is the formula weight of leucine, %Leu is the fraction of leucine in protein (0.073), C/Protein is the ratio of cellular carbon to protein (0.86), and ID is isotope dilution =2 (Kirchman 1993).

References

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Virus Like Particles Enumeration
(method used until ?? season)

General Discussion

Virus-like particles (VLP) are enumerated using the method of Hennes and Suttle (1995). Freshly collected samples are filtered onto 0.02 μm pore-size Anodisc 25 membrane filters (Whatman) and stained with the cyanine-based dye, Yo-Pro-1 (4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylmethyledene]-1-(3'-trimethylammoniumpropyl)-quinolinium diiodide; Molecular Probes, Eugene, OR). Virus densities were then estimated using epifluorescent microscopic direct counts (EMC) at a magnification of ×1250 using a Zeiss Standard 16 microscope with 100W Hg lamp and Zeiss P/N 09 combination filter set. At least 400 VLP are counted on each membrane filter.

Materials

0.02 μm filtered sterilized water
100 x 15 mm sterile, plastic petri dish
20 cc plastic syringe
25 mm 0.45 μm cellulose nitrate filters
9.0 cm Whatman #2 qualitative filter paper
Aluminum Foil
Cover slips
Disposable Acrodisc filters
Epifluorescence Microscope
Glass Slides
Whatman Anodisc 25, 0.02 μm Al_2O_3 filters

Reagents

Aqueous NaCl solution (0.3% wt/vol)
Glycerol (Spectrophotometry-grade)
DMSO
NaCN
Yo-Pro-1 Stock solution (supplied by Molecular Probes)

Procedure

1. Collect samples and process immediately without fixation or preservation.

2. Dilute stock solution of Yo-Pro-1 (1 mM Yo-Pro in a 1:4 solution of DMSO and H_2O) to 50 μM in an aqueous solution of 2 mM NaCN. Vortex well and store the 50 μM Yo-Pro working solution in the dark at 4^o C.

3. Place a series of 80 μl drops of Yo-Pro working solution in a 10 cm plastic Petri dish, spaced so that 25 mm filters may be placed on each drop without overlapping.

4. Label the Petri dish on the bottom side with sample ID's corresponding to each drop of Yo-Pro.
5. In the lid of the Petri dish, place a 9.0 cm Whatman #2 qualitative filter paper soaked with 2 ml of an aqueous 3% NaCl solution. Close the Petri dish containing the drops of Yo-Pro.

6. Clean filter funnels thoroughly with 0.02 μm filter-sterilized water (3 rinses of approx. 5 ml each)

7. Place backing filters, 25-mm 0.45 μm cellulose nitrate or equivalent, on filter towers and top with Whatman Anodisc 25, 0.02 μm Al_2O_3 filters. The Anodisc filters are very fragile so handle carefully with clean filter forceps, touching only the plastic support ring which surrounds each filter.

8. Replace clean filter funnels and add unfixed sample. For lake waters, volumes between 100-200 μl are sufficient. Cover filter funnels with clean Petri dish tops when not in use in order to avoid contamination.

9. Pipet sample onto the center of the Anodisc filter and bring sample volume to 2.0 ml with 0.02 μm filter-sterilized, *DI* water. Add this water drop-wise using a 20 cc plastic syringe and disposable 0.02 μm Acrodisc filter. Gently filter samples (< 15 kPa psi units???), and remove filter funnel once the sample has been completely filtered. Sometimes small drops of liquid remain around the edge of the Anodisc filter. Filter towers must be tapped vigorously, while vacuum is reapplied, to make sure that all the sample is filtered.

10. Remove Anodisc filters while still moist and place one filter (sample side up) on each drop of Yo-Pro in the previously-prepared Petri dish. Backing filters can remain on the towers and reused several times.

11. Replace the lid and cover the Petri dish with aluminum foil to exclude light. Incubate at 20° C for 2 days.

12. Following the incubation period, place the filter onto a clean filter tower and rinse twice with 800 μl of 0.02 μm filtered *DI* water.

13. Place the rinsed filter onto a glass slide; add a drop of spectrophotometry-grade glycerol to the center of the filter and a cover slip. Store prepared microscope slides in the dark at -20^{o}C. until processed. Dispose of Petri dishes.

14. Enumerate VLPs using an epifluorescence microscope (1000×) with an acridine orange filter set, and a 100 W Hg lamp.

15. Count at least 400 VLP on each membrane filter using a calibrated Whipple grid.

Note: Do not assume that the entire 25-mm diameter area of any filter is covered with particles of interest following filtration. The effective surface area for any combination of filter towers, funnels, and filters should be determined directly by microscopic measurement using a calibrated scale, graticule, or the Whipple grid itself.

References

Viral Production Determined by ^{3}H Thymidine Incorporation
(method used until ?? season)

General Discussion

Virus production is estimated by ^{3}H thymidine incorporation into TCA-insoluble <0.2 μm DNAse-resistant material by a method modified from Steward *et al*. (1992) and Fuhrman & Noble (1995). Samples are collected in 50 ml conical centrifuge tubes and ^{3}H-thymidine is added to a final concentration of 5 nM. Centrifuge tubes are incubated under *in situ* light and temperature conditions. Subsamples (7 ml) are collected through time for up to 48 h, and 5 ml of each is filtered through a 0.2 μm Acrodisc HT Tuffryn syringe filters (Gelman). Filtrate is split into duplicate 2 ml samples in 5 ml borosilicate glass test tubes. DNAse, RNAse, and micrococcal nuclease are added to the 2 ml samples and then incubated for 1 h. Next, 40 μl of 0.2 μm filtered formalin is added to each tube to stop enzyme activity and the samples are stored at 4° C until processed. Within 2 days, samples are divided into 900 μl subsamples and placed into 2 ml microfuge tubes on ice. A carrier solution (50 μg ml^{-1} each of DNA, RNA and BSA) is added to each replicate subsample. To each subsample, 300 μl of ice cold 20% TCA is added; one duplicate remains on ice while the other is incubated in a hot water bath at 100°C for 1 h. Hot samples are cooled on ice and all tubes are vortexed to resuspend precipitates. Samples are filtered through 25 mm HA Millipore filters; tubes are rinsed with 1 ml of 5% TCA and the rinse filtered. Empty microfuge tubes are discarded as isotope-contaminated solid waste. Filters and filter funnels are rinsed 3 times with 1 ml of cold 5% TCA. Remove funnels and rinse the edges of the filter 3 times with 1 ml of cold 5% TCA. Filters are placed in 20 ml glass scintillation vials, and 1 ml of 1 N HCl is added to each scintillation vial. Scintillation vials are heated (90-100° C) for 1 h to hydrolyze nucleic acids. Make sure the filters are dry. Once the samples are cooled to room temperature, add 5 ml of Cytoscint or Ecoscint (National Diagnostics) scintillation cocktail to each sample. Samples are counted using a liquid scintillation with quench correction.

Materials

0.02 μm Acrodisc (Gelman) filters
2.0 ml microfuge tubes
20 ml glass scintillation vials
25 mm HA Millipore filters
5 cc syringes
5 ml test tubes
50 ml conical centrifuge tubes
Ice

Reagents

^{3}H Thymidine
Carrier solution: containing equal volumes of DNA,RNA, and BSA at a concentration of 50 μg ml^{-1}.
Cytoscint cocktail: (or comparable scintillation cocktail)

Formalin
1 N *HCl*
Trichloroacetic acid (20% TCA)
TCA (5%)
TCA-insoluble < 0.2 μm DNAase resistant material

Procedure

1. Decant 40 ml duplicate samples into 50 ml conical centrifuge tubes (2 tubes per sample)

2. Add ^{3}H thymidine to a 5 nM final concentration in each tube (See bacterial production procedure for the calculation of ^{3}H thymidine volume added to each tube).

3. Collect 6 ml subsamples at 0, 6, 15, 24 & 48 h (10-5cc syringes per sample).

4. Filter 5 ml of each subsample thru 0.2 μm Acrodisc (Gelman) filters and split into duplicate 2-ml subsamples in 5 ml test tubes.
   (10 filters per sample)
   (20 tubes per sample)

5. Add nucleases to the 2-ml subsamples;
   10 μl DNase I at 1 unit μl^{-1} = 10 units × 20 subsamples = (200 units per sample)
   10 μl Rnase at 1 unit μl^{-1} = 10 units × 20 subsamples = (200 units per sample)
   10 μl Micrococcal nuclease at 5 unit μl^{-1} = 50 units × 20 subsamples = (1000 units per sample)

6. Incubate at 20° C for 1 h.

7. Add 40 μl of 0.2 μm filtered formalin to kill each sample.

8. Store samples at 4° C until processed.

9. Split into duplicate 0.90 ml subsamples and place in 2.0 ml microfuge tubes on ice (40 microfuge tubes per sample)

10. Add 100 μl of carrier solution containing 50 μg each of DNA, RNA & BSA (2 mg per sample)

11. Add 300 μl of cold 20% TCA to each subsample (2.4 ml TCA per sample)

12. Leave one duplicate on ice and incubate the other at 100^{o}C for 1 h.

13. Cool hot sample on ice for 10 minutes.

14. Vortex each tube to resuspend precipitates.

15. Place 25 mm HA Millipore filters onto filter base and replace tower. Filter sample under low vacuum (< 7 in Hg) and rinse microfuge tubes with 1 ml of 5% TCA (40 filters per sample).

16. Rinse filters and funnels 3 times with 1 ml of cold 5% TCA.

17. Remove funnels and rinse edges of filters 3 times with 1 ml of 5% TCA.

18. Place filters in 20 ml glass scintillation vials and add 1ml of 1N HCl.

19. Heat vials to 90-100^{o}C for 1 h to hydrolyze nucleic acids.

20. Allow vials to cool and add 5 ml of Cytoscint.

21. Count samples using a liquid scintillation counter with quench correction.

References

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Community Respiration Determined via the Electron Transport System
(method used until 0203 season)

General Discussion

This assay measures the oxidation of the coenzyme Q-cytochrome B complex, the rate-limiting step in the respiratory Electron Transport System (ETS). Coenzyme Q normally receives electrons from succinate or NADH and passes them to cytochrome-B_{556}. In this assay, the cells are lysed and adequate substrate provided, however cytochrome-B_{566} is inactivated. This results in the liberation of electrons, which reduce a tetrazolium salt (INT) to produce a pink formazin dye. The amount of the dye produced is proportional to the activity of the mitochondrial ETS. Electron transport system activity (ETS) is measured by the in vitro reduction of a tetrazolium salt (INT). Samples are collected with a 5L Niskin bottle during normal LTER limno sampling. Water is filtered through a Whatman GF/F filter, filters are homogenized in buffer, then assayed by a 2 h controlled temperature incubation resulting in the reduction of INT to the violet-colored formazan. Light absorbtion (Abs) is measured spectrophotometrically, and is directly proportional to the moles of electrons transferred through the electron transport system. Temperature correction used Ea=15000 cal mol^{-1} (Q10=2.66, taken as average literature value) for all stations. However, J. Priscu found Ea=9720 (Q10=1.89) for Bon E30 5m, 0-10 °C, on 9 Nov 90.

Materials

1 cm square cuvette
15 ml centrifuge cone tubes
30 ml Wheaton tissue homogenizer
47 mm GF/F filters
47 mm magnetic filter funnels
5 ml culture tube with screw caps
Assorted volumetric flasks (125, 250 and 500 ml)
DI rinse bottle
Electric drill
Filter manifold
Forceps
Glassine envelopes
Heat plate
Ice
Kimwipes
P1000 Gilson Pipetman and tips (2)
P5000 Gilson Pipetman and tips
Nicer bucket
Spectrophotometer
Vortex genie

Reagents

The volumes of reagents presented below are sufficient to perform at least 12 ETS assays (i.e., 12 complete limno runs; 3 ETS assays per West and East Bonney, Hoare, and Fryxell). However, adjust volumes appropriately based on different experimental design.

Homogenization Buffer: A 50 mM sodium phosphate buffer solution containing the following reagents:

$MgSO_4 \cdot H_2O$ (Magnesium sulfate monohydrate) **or**.....0.0064 g
$MgSO_4 \cdot 7H_2O$ (Magnesium sulfate heptahydrate).....0.0093 g
PVP (Polyvinylpyrrolidone).....0.7500 g
Stock A.....9.0 ml
Stock B.....114.0 ml
Triton X-100 .....1.0 ml

Bring to 500 ml with DIW and adjust to pH 8.0 with ≅ 10 N NaOH. **Note**: bring the buffer to ~ 475 ml with DIW, then add the Triton X-100, and continue diluting to 500 ml. This will help prevent unnecessary foaming of solution.

Stock A: Dissolve 0.05 moles monobasic sodium phosphate, NaH_2PO_4 (6.0 grams anhydrous or 6.9 grams monohydrate monobasic sodium phosphate) in DIW and bring to 250 ml. Final solution will be 0.2 N.

Stock B: Dissolve 0.1 moles dibasic sodium phosphate, Na_2HPO_4 (14.2 grams) in DIW and bring to 500 ml. Final solution will be 0.2 Normal.

Sodium Cyanide solution: A 0.1 M Sodium Cyanide solution.. Bring 0.6125 g of Sodium Cyanide: NaCN to 125 ml with DIW. Store at room temperature.

Substrate Buffer: A 50 mM Sodium Phosphate buffer containing the following reagents:

Stock A.....16.8 ml
Stock B.....184.0 ml
Triton X-100 .....1.6 ml

Bring to 800 ml with DIW and adjust to pH 8.0 with ≅ 10 N NaOH. Decant 52 ml aliquots into HDPE bottles and store at -20° C.

Note: Before preparing the *Substrate Solution*, remove 2 ml of *Substrate Buffer* and prepare the Turbidity Blank. This will leave 50 ml of Substrate Buffer for the *Substrate Solution*.

Substrate solution: A reagent containing 0.133 M disodium succinate; 0.835 mM NADH (di-sodium salt, f.w. = 709); 0.24 mM NADPH (tetrasodium salt, type III, f.w. = 833.4). Weigh the following dry reagents (15 replicates each) and place in 7-ml borosilicate sample vials and store in the dark at -20° C until the day of analysis.

Disodium Succinate (Succinic acid) .....1.0773 g
NADH .....0.0297 g
NADPH.....0.0100 g

Prepare Substrate solution immediately before use, by combining 1 vial each of the preweighed reagents with 50 ml of ice cold Substrate Buffer:

INT solution: A 2.5 mM solution of INT
$\left[2-(\text{p - iodophenyl})-3-(\text{p - nitrophenyl})-5-\text{phenyl tetrazolium chloride}\right]$

Bring 0.3792 g of INT to 300 ml with DIW. Decant 20 ml aliquots into 60 ml HDPE bottles and store in the dark at -20° C.

Termination Solution: A 1:1 mixture of 1 M Sodium Formate and Formalin. Bring 6.8 g Sodium Formate (f.w. = 68) to 100 ml with DIW; adjust to pH = 3.5 with 10 N HCl and combine with 100 ml formalin (37% Formaldehyde). Place solution in a 250 ml HDPE bottle and store at 4° C.

Procedure

1. Place 47 mm GF/F filters onto the magnetic filter base and replace the tower.

2. This assay requires a concentration > 1 µg of chlorophyll-*a* on the GF/F, thus large volumes of lake water must be filtered Table 6. Gently invert the clear 1-l HDPE bottle to thoroughly mix and decant 500 ml into a graduated cylinder. Filter this volume of water and repeat as necessary until the total volume is filtered.

Table 6. Volume of lake water filtered at each depth in each lake.

West Bonney		**East Bonney**		**Hoare**		**Fryxell**	
Depth (m)	Volume (ml)	Depth (m)	Volume (ml)	Depth (m)	Volume (ml)	Depth (m)	Volume (ml)
5	1000	4.5	1000	4.5	2000	4.5	1000
10	1000	5	1000	5	2000	5	1000
13	1000	10	1000	8	2000	6	1000
14	1000	13	1000	10	2000	8	1000
15	1000	15	1000	12	2000	9	1000
17	1000	18	1000	14	2000	10	1000
25	1500	25	1200	16	2000	11	1000
30	1500	30	1500	20	2000	15	1000

3. Once the total volume is filtered, gently remove the filter, fold in half (organic material inside), and place in a labeled glassine envelope. Store glassine envelope on ice, in the dark, until homogenization (< 1 h).

4. Prepare Homogenization Solution. Decant 49 ml of Homogenization Buffer and place in a HDPE bottle, add 1 ml of Sodium Cyanide Solution and mix solution thoroughly.

5. In an ice bath, homogenize sample filter in 2.5 – 3 ml of Homogenization Solution (2.5 ml for Lake Bonney and Hoare, 3.0 ml for Lake Fryxell) for 90 seconds ensuring the filter is completely homogenized. Adjust amount of Homogenization Solution to suit experimental design (0.5 ml of the extract will be needed for each replicate; allow at least 1 ml for loss in centrifuge).

6. Decant into a chilled centrifuge tube and rinse tissue homogenizer with two (0.5 ml) rinses of Homogenization Solution and decant into centrifuge tube. Place in ice bath until centrifuged.

7. Centrifuge on medium for 3 minutes at < 4° C, and briefly vortex samples, then centrifuge on high for 10 minutes at < 4° C. Promptly remove and place in ice.

8. Aliquot 0.5 ml of extract supernatant into three acid-washed culture tubes (labeled A, B, K). The tubes labeled A and B are replicate live treatments, and K is a killed treatment. Place the live treatment tubes in an ice bath.

9. Boil the killed treatment tubes for 10 minutes, and cool in an ice bath.

10. Prepare the Substrate Solution by adding the prewieghed dry reagents (Disodium Succinate, NADH, NADPH) to 50 ml of ice cold Substrate Buffer. **Note**: Begin to thaw one bottle each of the Substrate Buffer and INT Solution at room temperature while the ETS samples are filtering.

11. In each culture tube (A, B, K) combine the following reagents:

    Substrate Solution .....1.5 ml
    INT Solution .....0.5 ml

Prepare two additional control blanks to analyze with the samples. The sum of the Absorbance for these blanks will yield the total blank.

Abiotic blank.....0.5 ml Homogenization Buffer replaces 0.5 ml of sample
Turbidity blank.....2.0 ml of Substrate Buffer is used in place of the 1.5 ml Substrate Solution and 0.5 ml INT solution

Briefly vortex samples and incubate in an ice-water bath (0 – 2 ° C) for 1 h.

12. Stop the reaction by adding 0.5 ml of Termination Solution to each culture tube and briefly vortex.

13. Calibrate spectrophotometer with a *DI* blank and read absorbance at 490 nm using a 1 cm square cuvette. **Note**: Use the same cuvette for all readings and rinse with *DI* water between samples. Wipe the cuvette with a Kimwipe before analysis.

14. Community ETS (μmol O_2 l^{-1} h^{-1}) is calculated using the following equation:

$$\mathrm{ETS}\left(\mu\mathrm{mol\,O_2\,l^{-1}\,h^{-1}}\right)=\frac{\left(\mathrm{Abs_R}-\mathrm{Abs_K}\right)a\cdot b}{c\cdot t}$$

where Abs_R is the average absorbance of the replicate live treatments, Abs_K is the absorbance of the killed treatment, *a* is ratio of the volume of Homogenization Solution to the volume of lake water filtered (ml), *b* is the ratio of the final volume of reaction mixture in each cuvette to the volume of extract supernatant (ml), *c* is the stoichiometric extinction coefficient for formazan (31.8 Abs cm^{-1} μmol O_2^{-1}), and *t* is the incubation period (h). Community ETS is adjusted to ambient lake temperature (ETS_A) using the Arrhenius equation:

$$\mathrm{ETS_A}=\mathrm{ETS}\cdot e^{\left(\frac{\mathrm{Ea}\left(\left(\frac{1}{^{\circ}\mathrm{C_I}+273^{\circ}\mathrm{K}}\right)-\left(\frac{1}{^{\circ}\mathrm{C_A}+273^{\circ}\mathrm{K}}\right)\right)}{\mathrm{R}}\right)}$$

where Ea is the energy of activation (15,000 cal mol^{-1}, Q_{10} = 2.66), $°C_I$ is the incubation temperature (°C), $°C_A$ is the ambient lake water temperature at specific depth, R is a gas constant (1.987 cal mol^{-1} $°K^{-1}$).

15. A first-order relationship exists between ETS activity and respiratory capacity in aquatic microorganisms (e.g., Kenner and Ahmed 1975, Christiansen et al. 1980). In Lake Bonney, 44 % and 56 % of measured ETS activity is from bacterioplankton and phytoplankton, respectively (Takacs 1999). Using these percentages, in concert with published respiration:ETS ratios (Packard 1985), we derived the following relationship between community respiration and ETS for Lake Bonney:

$$\mathrm{Respiration}\left(\mu\mathrm{g\,O_2\,l^{-1}\,h^{-1}}\right)=\mathrm{ETS_A}\cdot 0.61$$

Notes from "Filtration Notes" Appendix:

Electron Transport System

- ETS samples are taken from the 1000 ml clear HDPE bottles. **Do not** use the amber HDPE bottle.

- Use either a 3 or 6 place manifold with 47mm magnetic filter towers and 47mm GF/F filters.

- Sample volumes vary with lake and depth. Decant 500 ml into a graduated cylinder and filter the entire 500 ml before decanting the rest of the sample in the graduated cylinder.

West Bonney		East Bonney		Hoare		Fryxell	
Depth (m)	Volume (ml)	Depth (m)	Volume (ml)	Depth (m)	Volume (ml)	Depth (m)	Volume (ml)
5	1000	4.5	1000	4.5	2000	4.5	1000
10	1000	5	1000	5	2000	5	1000
13	1000	10	1000	8	2000	6	1000
14	1000	13	1000	10	2000	8	1000
15	1000	15	1000	12	2000	9	1000
17	1000	18	1000	14	2000	10	1000
25	1500	25	1200	16	2000	11	1000
30	1500	30	1500	20	2000	15	1000

- Watch filter towers carefully, do not allow filters to go dry. Turn the valve on the manifold to the off position when there is still about 1mm of water left to go. Then remove the filter from the tower, fold in half (organic matter inside), and place in a labeled glassine envelope. Store the filters in a Nicer bucket containing ice until analysis.

Major ions by ion chromatography

(A new instrument is currently being used. Methods for the old instrument follow)

Sample Analysis

A Dionex DX-300 ion chromatography system (Dionex, Sunnyvale, CA, USA) is used for the major ion analyses. The system includes a gradient pump module (GPM), high-pressure injection valve with a 25 µl sample loop, Dionex conductivity detector (CDM-3), advanced computer interface (ACI), and automated sampler. The timed events and data collection are controlled by the Dionex AI-450 chromatography software for Windows. Only one ion chromatography system is used and is switched back and forth between anion and cation configurations. For the anions, a Dionex Ionpac AS4A-SC analytical column (4 × 250 mm) and AG4A-SC guard column (4 × 50 mm) are used along with an Anion Self-Regenerating Suppressor-1 (ASRS-1). The eluent is a 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$. The gradient pump flow rate was 2 ml min^{-1}. Background conductivity should be approximately 16 µS. For the cations, the Dionex Ionpac CS12 analytical column (4 × 250 mm) and CG12 guard column (4 × 50 mm) are used with a Cation Self-Regenerating Suppressor-1 (CSRS-1). The eluent is 0.02 M methanesulfonic acid (MSA). The eluent flow rate is 1.0 ml $minute^{-1}$ and background conductivity was approximately 200-250 nS.

Dissolved Organic Carbon by Total Organic Carbon Analyzer

(This method was used until the 0102 season. Since then samples have been run on the Shimadzu TOC-V series. Total nitrogen (TN) is also now analyzed during DOC analysis using the Shimadzu TNM-1 analyzer.)

General Discussion

The Dissolved Organic Carbon (DOC) procedure requires that samples be classified either as saline or fresh water. Saline samples (i.e., below 13 m Lake Bonney, below 9 m Lake Fryxell, Blood Falls) must be amuplated to allow complete wet-oxidation of organic carbon by sodium persulfate before analysis by TOC analyzer (OI analytical Model 700). Fresh water samples (Lake Haore, Lake Miers, Lake Joyce, Trough Lake, shallow waters of Lake Bonney and Lake Fryxell) may be run directly on the TOC analyzer without ampulization.

Materials

Dry Valleys Lab

Graduated cylinder
125 ml amber borosilicate glass bottles (acid washed w/ 10% HCL; combusted at 475° C for 4 h)
Green TFE-lined caps
Bell Jar filtering apparatus with bottle stands
25 mm polysulfone filter funnels
25 mm GF/F filters (combusted at 475° C for 4 h)
Eppendorf repeater pipet and tips

MCM Crary Lab

1000 ml Volumetric Flask (2)
Ampoule Purging and Sealing apparatus (OI Analytical Model 524)
Microwave for Lab use
P1000 Gilson Pipetman and tips
P200 Gilson Pipetman and tips
Precleaned ampules
TOC analyzer (OI analytical Model 700)

Reagents

Dry Valleys Lab

Hydrochloric acid (HCL): 6N

MCM Crary Lab

Organic free water
Potassium acid phthalate (KHP)
Sodium persulfate
Stock Organic Carbon solution: Dissolve 2.1254 g anhydrous potassium biphthalate ($C_8H_5KO_4$) in carbon-free water and dilute to 1000 ml; 1 ml = 1 mg carbon.

Procedure

Below steps to be conducted in Dry Valleys Lab

Sample Collection

1. The filtrate produced from the chlorophyll-a filtration (Section 3.1) is collected for DOC analyses.

2. Gently invert the 1000 ml amber HDPE bottle, thoroughly mixing the sample, and decant 100 ml of sample into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the filter base and replace filter tower. Collect 100 ml of the filtrate in an acid-washed, combusted 125 ml amber borosilicate glass bottle. Filter sample under low vacuum (< 7 in Hg). Do not rinse the filter tower during this step. Only rinse the filter towers between sample depths.

4. Carefully remove the bell tower and cap the amber bottle.

5. Once all of the DOC samples are filtered, preserve each 100 ml sample with 1.0 ml of 6N hydrochloric acid using the Eppendorf repeater pipet. If less sample volume is filtered, adjust the volume of hydrochloric acid accordingly. Store the samples in the dark at 4° C until analysis.

Below steps to be conducted in MCM Crary Lab

Sample Analysis

1. Open precleaned ampoules.

2. Add 2ml of sample to ampoule.

3. Add 200 µl of 5% HCL (prepared with Organic free water) to ampule.

4. Purge using air on Purging and Sealing unit for approx. 5 minutes.

5. While maintaining purge, add 1 ml of 100 g/l Sodium Persulfate solution (prepared as directed by TOC manual and purged with nitrogen) to ampoule. Do this step individually just before sealing.

6. While maintaining positive oxygen flow in the ampoule, seal the ampoule.

7. Bake the sealed ampoules at 105 °C for about 8 h to drive the reaction to completion.

8. Prepare a standard curve from the stock organic carbon solution (10 – 50 µg C) and a blank.

9. Analyze samples and standard curve with the TOC analyzer (Consult the TOC manual for injection procedures).

10. Use the regression equation of the standard curve and calculate the DOC (mg/l) for each sample.

Notes:

1. Samples with greater than 50 μg C must be reanalyzed with an appropriately reduced sample volume.

2. Some samples will have high carbonates and fizz when acid is added. Add acid slowly so as not to loose sample.

3. It is important to maintain a pure oxygen atmosphere inside the ampoule. This will ensure that atmospheric CO_2 does not intrude, and will also aid in complete oxidation of the organic carbon.

4. Do not allow the sample to sit for long periods of time between procedure steps 10 and 11, as oxidation of carbon within the sample will begin and some carbon will be lost as CO_2.

Particulate Carbon and Nitrogen Analysis by Elemental Analyzer (Filters and Sediment)

(Samples are currently being analyzed with a CE Instruments Flash EA 1112 (ThermoQuest) elemental analyzer. The method outlined below was used until the 0203 season.)

General Discussion

This method is used in the analysis of carbon and nitrogen on filters (water) and sediment samples that have been prepared using ASA Analytical Services method described below. Samples are analyzed with a Carlo Erba NA 1500 elemental analyzer which flash combusts at 1800° C. Combustion gases pass through a catalyst converting all carbon and nitrogen combustion products to CO_2 and N_2. The gases are then separated by gas chromatography and detected by a thermal conductivity detector.

Materials

Sampling Hut

1000 ml Amber HDPE bottles

Dry Valleys Lab

Graduated cylinder
25 mm GF/F filters combusted at 475° C for 4 hrs spread out on aluminum foil that will serve as a wrapper after combustion.
25 mm Polysulfone filter towers
Vacuum pump
Filter forceps
Aluminum weigh boats
Zip Lock Bags

MCM Crary Lab

96-well plate: polystyrene
Cork borer (1 cm)
Elemental analyzer: Thermofinnigan EA 1112 Flash
Filter apparatus
Forceps: Assorted sizes and types
Glass plate
Metric ruler
Micro spatula
Micro-balance
Pre-formed silver cups (Type H)
Pre-formed tin cups (Type A)
Quartz combustion tube
Quartz wool

Interferences

1. The elemental analyzer is very sensitive and will detect organic carbon and nitrogen in fingerprints. Handle all samples and standards with forceps to avoid contamination.

2. Ensure that the preparation area is clean of any standard when working with samples.

Reagents
Acetanalide: standard grade, pre-dried at 70 °C and desiccated (% C = 71.09, % N = 10.36). Reagents for combustion and reduction are described in the instrument manual

Procedure
Sample Collection
1. Fill a 1000 ml HDPE amber bottle with 1000 ml of sample from the Niskin bottle. Place in cooler for transport

Below steps to be conducted in Dry Valleys Lab
2. Particulate organic carbon and nitrogen lake water samples are taken from the 1000 ml amber Nalgene bottle. Gently invert the bottle, thoroughly mixing sample, and decant 500 ml into a graduated cylinder.

3. Place a combusted 25 mm GF/F onto the polysufone filter base, replace tower, and filter the sample under low pressure (<10 in Hg). **Note**: The filter tower will only hold 200 ml of sample, therefore, continually top off the sample until the entire volume is filtered. Furthermore, many of the Lake Bonney samples will take between 6-12 h to filter, thus plan accordingly.

4. Once the entire volume is filtered, place the filter (organic matter up) in a labeled aluminum weigh boat, and dry at room temperature for 12 h. Following desiccation, stack all of the aluminum weigh boats together; place an empty one on top, ensuring the bottoms are clean; tape together and store frozen in a zip lock bag. Include a GF/F filter from the same packet of combusted filters used for the samples to be analyzed as a blank.

Below steps to be conducted in MCM Crary Lab
Sample Analysis (filters)
1. Using forceps, place sample filter on a clean glass plate.

2. Place the filter into one compartment of a 24-well plate.

3. Place the 24-well plate, with the top removed, into a dessicator containing ~50 ml of concentrated HCl. Allow the filters to fume overnight to remove inorganic carbon. Samples may be stored at -20°C or processed further at this point.

4. Using forceps, place the filter section on a type A tin cup that has been unfolded. Be careful not to puncture holes in the foil; fold the foil over and totally cover the filter section. Keep folding the foil as to encapsulate the section to a size of, approximately, 3 mm square. The sample must fit into the sample chamber of the Carlo Erba NA 1500.

Note: This step is largely technique based and, if possible, should be demonstrated by an experienced analyst.

5. Place the encapsulated sample into a cell of a 96-well plate. Samples may be analyzed immediately or stored at -70° C.

Sample Analysis (sediment)

1. Ensure that sediment samples are as homogeneous as possible. Handle all sample cups with forceps to prevent contamination from your skin.

2. Pre-formed silver cups must be cleaned before use.
   Rinse a 1000 ml beaker with 50 ml of acetone.
   Pour silver cups into beaker.
   Add enough acetone to beaker to cover silver cups and mix with a stir rod to ensure all surfaces are covered.
   Pour off acetone.
   Put cups into a tin foil boat and place into a 70° C drying oven overnight.
   Put individual cups into cells of a 96-well plate, cover and tape closed to minimize exposure of cleaned cups.

3. Weigh out 15-20 mg of dried sediment (105 °C for >24 h) into a clean, pre-formed silver cup.

4. Place cup into a cell of a new 96-well plate (record cell #). With the lid of the plate removed, place it into a dessicator, containing ~100 ml of concentrated HCl, overnight.

Note: Do not leave silver cups in an acidic environment for more than 14 h. The acid will cause the cups to become brittle and they will break and crack when they are manipulated.

5. Remove the 96-well plate. Carefully crimp the tops of the cups and compact the them into a, 3 mm or smaller cube.

Note: This step is largely technique based and, if possible, should be demonstrated by an experienced analyst.

6. Return the sample to the 96-well plate. These samples should be analyzed as soon as possible as traces of acid will continue to react with the silver cups.

Instrument Preparation

1. It is important to wear gloves when handling and packing the combustion tube. Fingerprints can cause the tube to crack or shatter causing injury.

2. Use combustion tube diagram, in the instrument manual, and a ruler to measure and pack the column, with copper wires and tungstic anhydride, using the quartz wool to separate

catalysts. **Note**: Tungstic anhydride comes prepackage in the correct amount needed for packing.

3. Insert the packed tube into the instrument, from the top side of the instrument, being careful not to chip the end of the tube on the inside of the furnace. Grasp the bottom of the tube and carefully place the locking nut, metal spacer and O-ring onto the tube as shown in the instrument manual. Seat the locking nut on the coupling and tighten hand-tight.

4. While still holding onto the tube, place the O-ring onto the top of the tube. Seat the autosampler onto the O-ring and tighten the locking nut hand-tight.

5. Proceed with instrument set-up and perform a leak check to ensure a helium tight seal.

Note: The combustion tube catalysts have a limited sample life. When CCV's start to approach the lower limits of acceptable range, or the tube is full, a new tube must be prepared.

Instrument set up

1. Open the valves to the air, oxygen and helium cylinders.

2. Turn the main power switch on.

3. The instrument parameters should be set according to the instrument operating manual. Once set, they should not have to be changed.

4. If the combustion tube has been changed, perform a leak test.

5. Allow the combustion chamber to come to stand-by temperature and turn on the sample purge valve.

6. Turn the instrument off of stand-by and allow to come to operating temperature.

7. Turn on the instrument computer and boot up the HP Chemsoft software package.

Leak Test

1. Turn the carrier gas and detector gas regulators (the regulators on the instrument panel, not the regulators attached to the cylinders) below 100 psi.

2. Cap off the carrier and detector outlet lines with the provided plugs.

3. Carefully, so as not to overshoot, adjust each regulator (instrument panel) to 100 psi and allow the pressure to equilibrate for a few seconds.

4. Turn the carrier and detector gas regulators (instrument panel) down several turns.

5. Observe the pressure gauges for 60 seconds and ensure that there is no drop in pressure. If a drop in pressure is observed, check combustion tube fittings and repeat the leak test. If a leak is still present, refer to the instrument technical manual for a more thorough test procedure that will narrow and identify the leak.

6. Remove plugs from outlet lines and adjust the regulators back to manual specifications.

Setting Instrument Flow rates

1. Set-up the flow-meter, as described in the flow-meter manual.

2. Attach the flow-meter hose to the carrier gas outlet and measure the flow. If the flow rate is off, according to the instrument manual, adjust the carrier gas pressure regulator (instrument panel) until the correct flow is achieved.

Note: If the pressure needed to achieve proper flow rates is above 100, there is a block in the carrier line. A common cause of this is saturated drying material in the water trap.

3. Repeat the flow check on the detector and oxygen lines and adjust as necessary.

Instrument Calibration

1. Calibration standards for filter samples are made in tin cups. For sediment samples, silver cups are used.

2. Using forceps, place a single cup on a micro-balance and tare. Remove the cup from the balance and place on a clean glass plate.

3. Use the micro-spatula to assist in weighing between 25 and 550 μg of acetanalide standards. Record the weight.

22. Make a total of six standards, ensuring the weights are spread out evenly between 25 and 550 μg.

5. Crimp and fold three cups to be used as blanks and place in the 96-well plate.

6. Load the auto sampler, starting with the three blanks and ending with the high standard.

7. Enter the blanks and standard amounts into the sample table of the chemsoft program, as described in the software manual, and start the run.

23. Determine the theoretical yield of carbon and nitrogen for each standard. Acetanalide contains 71.09% C and 10.36% N. Multiply each standard weight by the fraction of C and N in the standards to determine the expected μg of each C and N respectively (25 μg acetanilide contains 17.77 μg C and 2.59 μg N; 550 μg acetanilide contains 390.99 μg C and 56.98 μg N).

9. Plot the peak area vs. the expected μg for each analyte and ensure a linear fit with a correlation of 0.995 or better, to validate calibration.

Quality Control

1. Analyze one blank cup per 20 samples. Ensure that the blank measures at or below the instrument detection limit to check for instrument drift.

2. Analyze one mid-range standard per 20 samples. Ensure a percent recovery between 90 - 110 %, when compared to the standard curve, to continue analysis. If the recovery is not within this range, check instrument operation.

3. Analyze one duplicate sample per 20 samples. Ensure a coefficient of variation of between 70 - 130 % to confirm precision.

Instrument Shut Down

1. Turn the sample purge flow off.

2. Turn the instrument to stand-by and wait for the combustion chamber to reach stand-by temperature.

3. Turn the gas flow at the gas cylinders off.

4. Turn the main power to the instrument off.

References

Carlo Erba Elemental Analyzer, Instrument Manual.
Carlo Erba Elemental Analyzer, Technical Manual.

Ammonium Analysis Using a Manual Method
(Samples are currently analyzed for Ammonium using the Autoanalyzer)

General Discussion

Materials
P10 ml Gilson Pipetman
25 ml test tubes (acid soaked)
Assorted volumetric flasks (100, 250, 1000 ml)

Reagents
Alkaline citrate solution: Dissolve 50 g of analytical reagent grade trisodium citrate dihydrate and 2.5 g of analytical reagent grade sodium hydroxide in 250 ml of *DI* water.

Ammonium stock solution (1 mM): Bring 0.1070 g oven-dried (>4 h at 100 °C) NH_4C1 to 2000 ml with *DI* water. This will make a 1 mM solution. Store at 4 °C.

Oxidizing solution: Mix 100 ml of the alkaline citrate solution with 25 ml of sodium hypochlorite solution. Prepare this mixture fresh daily.

Phenol-ethanol solution. Dissolve 10 g of analytical reagent grade phenol in 100 ml 95% ethanol**. Handle with caution.**

Sodium hypochlorite solution: Use analytical reagent grade sodium hypochlorite solution (NaOCL) or a household bleach. **Note**: These solutions loose chlorine over time and may have to be replaced often.

Sodium nitroferricyanide solution: Dissolve 0.5 g of analytical reagent grade sodium nitroferricyanide in 100 ml of *DI* water. Store at 4° C in a dark bottle wrapped with aluminum foil, replace monthly.

Procedure
1. Thoroughly rinse acid soaked 25 ml test tubes and rinse 6× with *DI* water.

2. Add exactly 10 ml of *DI* water to each of the first ten tubes and then add the appropriate volume of ammonium stock solution (Table 12) and vortex. These tubes will serve as standards:

Table 12. Volume of stock ammonium solution use to create standard solutions.

Tube #	Volume of 1 mM stock added (ml)	Final Standard. Concentration (μM)
1	0	0
2	0	0
3	0.05	4.975
4	0.05	4.98
5	0.10	9.901
6	0.10	9.901
7	0.20	19.608
8	0.20	19.608
9	0.30	29.126
10	0.30	29.13

3. Fill the remaining tubes with 10 ml of sample. For saline samples requiring 1:10 dilution (See Dilutions on Page 94), add 9 ml of *DI* water to 1 ml of sample.

4. Add 0.4 ml of the phenol-ethanol solution to each tube and vortex.

5. Add 0.4 ml of sodium nitroferricyanide solution to each tube and vortex.

6. Add 1.0 ml of oxidizing solution to each tube and vortex.

7. Place all the tubes in total darkness; let color develop for a minimum of 2 h.

8. Calibrate spectrophotometer to read zero absorbance (100% transmittance) against *DI* water. Measure the absorbance of the calibration standards and samples at 640 nm. Use the same cuvette for all readings. Rinse cuvette with *DI* water between samples.

24. Use the standard curve regression equation to calculate NH_4^+ values (μM) for each sample.

Nitrate Analysis by Spongy Cadmium Reduction (Manual Method)
(Samples are currently analyzed for Nitrite using the Autoanalyzer)

General Discussion

In this assay, the concentration of nitrate is determined by cadmium reduction to nitrate followed by a diatozation-coupling reaction resulting in the formation of a red azo dye, which can be analyzed colorimetrically. Nitrate concentration is assumed to be equal to the difference between nitrate concentration of the sample before and after nitrate reduction. This procedure is based on "An Alternate Method for Nitrate Reduction by Shaking with Spongy Cadmium" (Jones, 1984) with some modifications adapted from the copper-activated Cadmium column reduction method found in Standard Methods (APHA 85). The method of nitrate reduction presented by Jones offers certain advantages over the copper-activated cadmium column method. It eliminates the problems of unequal reduction efficiencies among multiple reduction columns and prevents the gradual decline in reduction efficiency associated with cadmium columns. Also, because the spongy cadmium is cleaned and acidified each time it is used, the danger of sample contamination is reduced greatly.

Materials

25 ml standard test tubes
50 ml pyrex screw top test tubes
Assorted volumetric flasks
P10 ml Gilson Pipetman and tips
Shaking Table
Spectrophotometer (providing a light path $\geq$ 1 cm and reading at 543 nm)
Vortex

Reagents

Cadmium Sulfate Solution: Dissolve 20 g of $CdSO_4$ in 100 ml of *DI* water (20% $CdSO_4$ w/v)
6N Hydrochloric Acid
Zinc metal sticks: Sticks should be roughly 10 cm long and 1 cm in diameter, with purity near 99.9995%. However, use of zinc that is slightly less pure should have no detrimental effect on the outcome of the assay. Zinc does not play a direct role in reduction of the sample; furthermore, the spongy cadmium is rinsed thoroughly with deionized water after formation, thus greatly reducing the possibility of contamination through zinc impurities.
Spongy Cadmium: Place two sticks of zinc metal in 80 ml of cadmium sulfate solution overnight. Reaction of two sticks with 80 ml of solution in a slender glass container will produce roughly 15 g of wet spongy Cadmium ($CdSO_4$ is nearly completely consumed in this reaction.) Using a plastic spatula or pipet tip, separate the cadmium precipitate from the zinc sticks. Remove the zinc sticks and store. Acidify the solution with a few drops of 6N HCl and drain it from the precipitate. Cover the cadmium precipitate with 6N HCl and stir, breaking up any large aggregates. Drain the acid, then rinse the precipitate with *DI* water until pH is above 5 ($\approx$ 10 times) and store under DIW. It is essential to keep the cadmium

wet at all times. After the reduction step is completed, reactivate the cadmium by repeating the HCl wash and *DI* rinse.

N-1-napthyl)-ethylenediamine dihydrochloride (NED dihydrochloride): Dissolve 500 mg NED dihydrocholoride in 500 ml *DI* water. Store in a dark bottle. Replace monthly or upon the development of a dark color.

Ammonium Chloride-EDTA buffer solution: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 ml *DI* water. Bring pH to 8.5 with concentrated NH_4OH (under fume hood) and dilute to 1000 ml. This solution is less prone to interference by metals and turbidity in the water sample than the buffer used by Jones (1984). Isolate this reagent from those used for ammonium determination.

Sulfanilimide solution: Add 50 ml concentrated HCl to 300 ml *DI* water. Add 5 g sulfanilimide and vortex well, dilute to 500 ml. The solution is stable for several months.

Stock Nitrate solution: NO_3^--N (1000 mg l^{-1}). Dissolve 7.218 g of KNO_3 (dried in an oven at 105° C for 24 h) in water and dilute to 1000 ml.

Standard Nitrate solutions: (10 mg l^{-1}). Dilute 10.0 ml of nitrate stock solution to 1000 ml with *DI* water. Prepare calibration standards in the range of 0-10 mg NO_3^- - N l^{-1} by diluting the following volumes (5, 15, 25, 35, 45 ml) to 50 ml with *DI* water

Spiking Nitrate solutions: (50 mg l^{-1}). Dilute 5.0 ml of each stock solution to 1000 ml with *DI* water.

Procedure

1. Pipet 10 ml of each sample into an acid-washed 50 ml screw-top test tube.

2. Add 10 ml EDTA buffer solution to each sample. A bottle-top dispenser is a convenient way to distribute the buffer to the tubes. **Note**: Samples that have NO_3^-+NO_2^- concentrations above 30 μM should be diluted. Lake Bonney samples below 15 m are diluted 1:10.

3. Prepare calibration standards in the same manner as samples.

Reduction of NO_3^-

1. Add approximately 0.5 g spongy cadmium to each sample. To avoid possible metal interference, use a plastic spatula (or a plastic pipet tip cut lengthwise) to transfer cadmium. Because the cadmium will be present in excess, absolute precision in weighing is not necessary. Weighing a single cadmium sample and then estimating weights for subsequent samples is sufficient to ensure consistency in reduction.

2. Screw caps firmly on test tubes and place horizontally on shaker table. Shake for approximately 90 minutes at 100 RPM. Pipet 10 ml reduced sample into 25 ml test tube.

Color development and measurement

1. Add 0.4 ml sulfanilamide reagent to each reduced 10 ml sample and vortex. Let the reagent react for 2-8 minutes.

2. Add 0.4 ml of NED-dihydrochloride solution. Vortex immediately.

2. Allow color to develop for at least 20 minutes but not more than 2 h.

3. Calibrate spectrophotometer to read zero absorbance (100% transmittance) against *DI* water. Measure the absorbance of the calibration standards and samples at 543 nm. Use the same cuvette for all readings. Rinse cuvette with *DI* water between samples.

4. Use the standard curve regression equation to calculate NO_3^- -N values (mg l^{-1}) for each sample.

Dissolved Oxygen by Mini-Winkler Titration

(In 0506 we started using a pre-maid sodium thiosulfate solution. The following method includes standardization using a bi-iodate standard as was done prior to 0506.)

General Discussion

Materials

Sampling Hut

Benchcoat
Kimwipes
P1000 Gilson Pipetman and tips (2-one for each reagent)
Vinyl gloves (at least 3 pair)
Glass scintillation vials (20 ml vials with HDPE cone caps)
Serum/Scintillation vial carrier
Plastic scintillation tray
Ziplock bag for waste

Dry Valleys lab

Benchcoat
Kimwipes
P1000 Gilson Pipetman and tips (3-one for each reagent)
DI squirt bottle
Eyedropper
Forceps
Gilson micro-buret (2 ml)
Lamp or good sunlight
Latex/vinyl gloves (at least 3 pairs)
Magnetic stir plate
Micro stir bar
Mini beakers (10 ml)
Waste bottle
Ziplock for lab waste

Reagents

Sampling Hut

Alkaline-iodide-azide solution: In a volumetric flask bring the following reagents to 250 ml using *DI* water:

- 2.5 g sodium azide (NaN_3)
- 25 g potassium iodide (KI)
- 80 g sodium hydroxide (NaOH)

Manganous solution (Mn^{++}): In a volumetric flask bring 100 g of $MnCl_2 \cdot 4H_2O$ to 250 ml using *DI* water.

Dry Valleys Lab

Alkaline-iodide-azide solution (see above)
Manganous solution (Mn^{++}) (see above)

Phosphoric acid (H_2PO_4): Concentrated

Sodium thiosulfate titrant ($Na_2S_2O_3 \cdot 5H_2O$): In a volumetric flask bring 0.620 g of $Na_2S_2O_3 \cdot 5H_2O$ to 250 ml using *DI* water.

Standard potassium bi-iodate solution ($KH(IO_3)_2$): In a volumetric flask bring 0.03256 g of $KH(IO_3)_2$ to 100 ml. However, it is easier to prepare a ×10 concentrated solution and immediately before titration bring 10 ml of the concentrated solution to 100 ml in a volumetric flask with *DI* water.

Starch solution (1%): Dissolve 1 g of laboratory –grade soluble starch in 100 ml of hot *DI* water.

Procedure

Sample Collection

1. Place the Niskin bottle tubing to the bottom of the 20 ml scintillation vial. Allow the water overflow, displacing the initial sample volume 2x (about 5 seconds). Carefully remove the tubing, keeping turbulence to a minimum, making sure the vial is completely filled with no air bubbles.

2. Fix the sample by adding the following reagents (directly into sample):
   0.9ml of the Mn^{2+} solution
   0.9ml of the alkali-iodide-azide solution

3. Cap the sample and invert several times to ensure mixing. Use benchcoat on the table top and kimwipes to mop up the excess fluid that is displaced.

4. Place sample into the Serum/Scintillation vial carrier for transport.

Below steps to be conducted in Dry Valleys Lab

Sample Analysis

4. Prepare each sample for titration by adding 1 ml of phosphoric acid. Invert each sample several times and wait for precipitate to dissolve.

2. Dilute the concentrated potassium bi-iodate solution. Bring 10 ml of the concentrated solution to 100 ml in a volumetric flask using *DI* water.

3. Prepare a standard by filling a 20 ml scintillation vial to the brim with the diluted potassium bi-iodate solution, and add the following reagents:
   0.9 ml of the alkali-iodide-azide reagent.
   1 ml of phosphoric acid.
   Cap the sample and invert several times to mix.

4. Sample titration: Place a kimwipe on the stir plate and position the plate such that it is in direct light (a desk lamp may be used) to easily observe colorimetric change.

5. Withdraw 2 ml of sodium thiosulfate into the microburet. **Note:** To minimize contamination of the thiosulfate, fill a scintillation vial with thiosulfate and use instead of the reagent bottle.

6. Pipet 1 ml of sample (or standard) into a 10 ml disposable beaker, and place a micro stir bar into the sample.

7. While keeping the tip of the microburet in the sample, slowly titrate to a pale straw yellow color; add 1-2 drops of starch solution (heat starch solution prior to addition to make sure starch is in solution), this will turn the sample dark blue; then titrate to endpoint (first disappearance of blue color). Place a mini beaker containing 2 ml of *DI* water along side the sample beaker. This will provide a reference color during sample titration. **Note**: If the sample does not turn dark blue, the starch may have precipitated. Resuspend the starch by heating solution (hot, but not boiling).

8. Record the volume of titrant, and repeat titration. Titrate each sample at least 2 times and the standard at least 4 times. Variation among the replicates should be less than ± 0.5%.

Note: After each titration, rinse the buret with *DI* water (into a waste bottle), and wipe dry with a kimwipe. Then refill with the thiosulfate solution, and wipe the buret dry again. Also, pour the titrated sample into the waste bottle; rinse the mini beaker with *DI* water and wipe dry.

9. Determine oxygen concentration as follows.

$$\text{Dissolved Oxygen}\left(\text{mgO}_2\,\text{l}^{-1}\right) = \frac{(\text{ml titrant})(\text{M}_{\text{thio}})(8000)}{(\text{ml sample titrated})\left(\frac{\text{ml of bottle} - 1.8}{\text{ml of bottle}}\right)}$$

where M_{thio} is the molarity of thiosulfate, and 8000 converts thiosulfate equivalents to oxygen equivalents and ml filtered to liters.

References

Carpenter, J.H. 1965. The Chesapeake Bay Institute technique for the Winkler Dissolved Oxygen method. Limnology and Oceanography. 10(1):141-143.

In-situ Dissolved Oxygen and Temperature Profiles

(Method used until 99-00 season)

General Discussion

Dissolved oxygen and temperature profiles are collected from the Weatherport sampling hole using an oxygen sensitive membrane polargraphic electrode.

Materials

YSI Model 58 dissolved oxygen meter
YSI Model 5739 dissolved oxygen probe with a 50 m cable

Procedure

1. Before each sampling, check the condition of the batteries in the meter, the connection between the probe and cable, and replace the probe membrane and solution. Use a high sensitivity membrane and half saturated KCl solution. Always store the probe in a water-saturated air environment with its protective cap in place.

2. Turn the meter on and place the probe, with its protective cap in place, directly above the piezometric water level inside the hole. Allow the probe to polarize and reach temperature equilibrium (~15 minutes).

3. To calibrate the instrument, set the SALINITY scale to 0 ppt, adjust the ZERO knob to read zero, and set the calibration knob to read the correct dissolved oxygen value based on the probe temperature and local atmospheric pressure (Consult the dissolved oxygen saturation curve on the back of the meter). Do not change the SALINITIY scale during measurements. Even though the deep water of Lake Bonney exceeds 40 ppt, the salinity scale is set to zero for comparative purposes.

4. Lower the probe to the desired depth and slowly agitate the probe in an up and down motion (~ 2 cm). Allow the probe to reach equilibrium and record the temperature, dissolved oxygen (mg l^{-1}), and % saturation. **Note**: The temperature value will quickly stabilized, however, the dissolved oxygen may take **at least 10 minutes** to reach equilibrium at each depth. Record data at 1 m increments down the water column. Do not allow the probe to come in contact with the benthos.

Underwater Time Series PAR Logged During Primary Production Experiment (Using LI-1000)

General Discussion

Materials

Licor LI-1000 Data logger with 30 m cable
Licor LI-193SA spherical quantum sensor
Licor LI-190SA quantum sensor
Leveling fixture
Lowering Frame
Tarpaulin (opaque)

Procedure

1. - Clear the memory of the LI-1000 by pressing "FCT/ON" and scrolling with the down arrow until you see "Clear Ram." Press Enter and say yes. Exit by pressing "FCT/ON."
   - Set the date and time by pressing "TIME" and entering the current date and time.
   - Configure the LI-1000 to record light data on channels 1 (underwater sensor) and 2 (incident sensor); enter the appropriate sensor multiplier for each channel (See LI-1000 Manual and multipliers attached to each sensor).

2. Configure the LI-1000 to Log Mean PAR values every 10 minutes:
   1. CFG — mode is LOG
   2. range — A (autorange)
   3. label — UM (μmol photons s^{-1} m^{-2}) (this can be left blank for easier data processing)
   4. multiplier — **Correct Multiplier on PAR sensor**
   5. per (logging period) — 10
   6. interval — 5 (LICOR automatically chooses 5 seconds for a 10 min period)
   7. reset — **Time the PAR logging should begin**

8. threshold (thr)	-1.000E+09
9. store	Mean
10. tcoff	0.0036 (this parameter is only shown if "store" is set to "Int."
11. min/max	no
12. time stamp	yes (this parameter is only shown if "min/max" = yes.

3. The time series logged data is recorded inside the weatherport sampling hole. Once all of the water samples have been collected, lower the underwater sensor frame to a depth of 10 m (7 m at Lake Fryxell) below the piezometric water level and secure to the winch frame. Place the ambient sensor frame as far away from the weatherport as possible and attach both cables to the appropriate channels on the LI-1000. Make sure you give yourself enough time, once the **reset time** is programmed, to position the sensors and data logger.

4. Allow the LI-1000 to log for a 24 h period, wake up the instrument by pressing the FCT/ON button and then turn the instrument off. **Note**: Due to logistic and time constraints it is difficult to immediately start PAR logging once the PPR samples are placed in the incubation hole.

5. Download the recorded data to a computer or ZIP disk. Follow the instructions provided below (http://env.licor.com).

Using Windows®95 HyperTerminal to Download From the LI-COR® LI-1000 (during the 0405 season, we used Tattle Term instead of Hyperterminal – see below for these instructions)

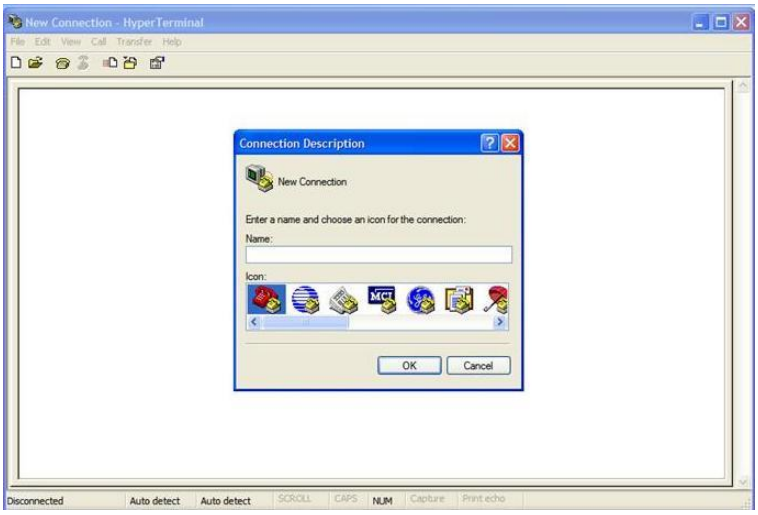
If you use a PC with Windows 95 software, there is an easy way to retrieve data from the LI-COR LI-1000 without having to use the 1000-90 communications software program available from LI-COR. HyperTerminal (found in the accessories folder of Windows®95) will work just fine.

Steps for setting up HyperTerminal

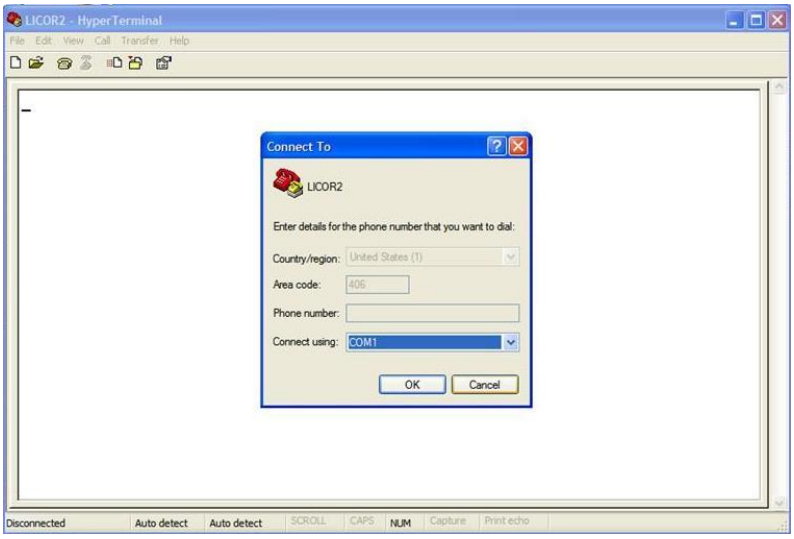
1. Connect the LI-1000 to one of your PC's serial connections using either the 1000-03 cable with 25-pin connector or the 1000-09 cable with 9-pin connector depending on your PC's requirements

2. Open the HyperTerminal folder and double click on Hypertrm.exe. (If a screen appears prompting you to install a modem, select No.). Open Hyperterminal (located in the "Communications" folder under "Accessories": start → programs → accessories → communications → Hyperterminal)

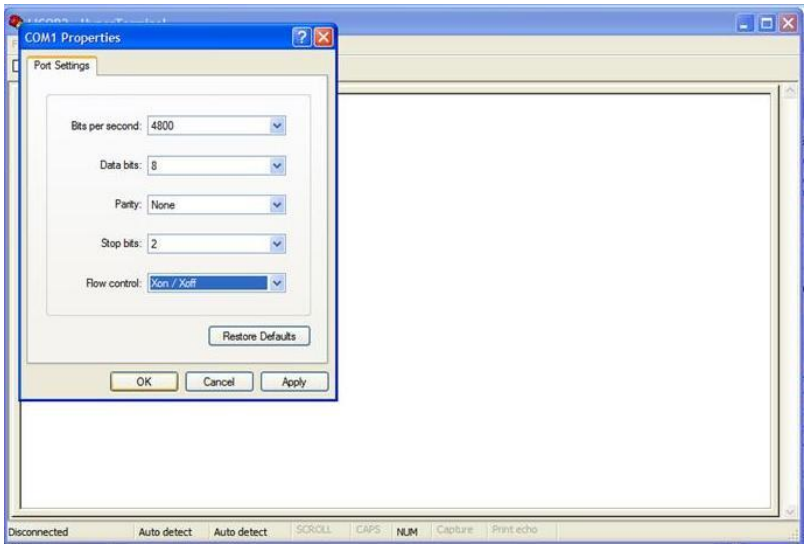
3. Select a name for the connection, such as LI-1000 and then select OK. (**Note**: HyperTerminal will automatically add an .ht extension to the filename, so it will appear as LI-1000.ht the next time HyperTerminal is opened.)



4. Select the appropriate connector for your connection under "Connect using" (Direct to Com 1, Direct to Com 2, or…) and select OK.

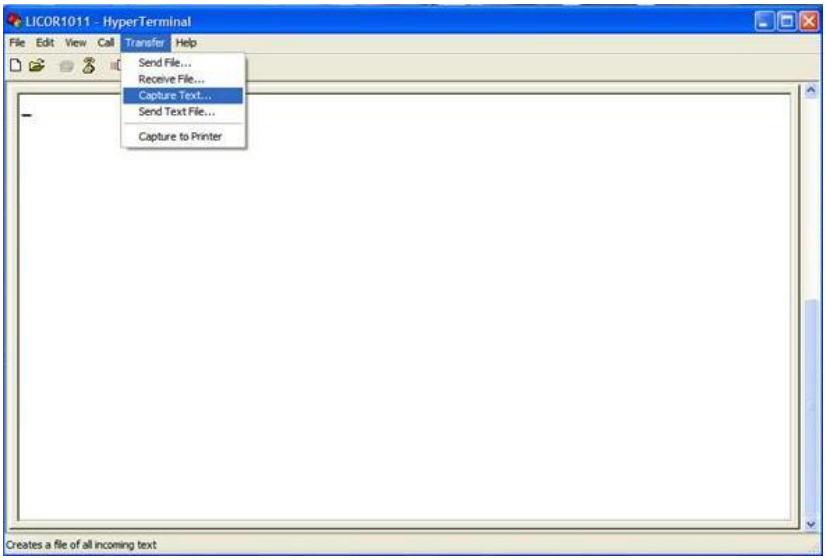


5. Change the communications settings to the following: Bits per second = 4800, Data bits = 8, Parity = None, Stop bits = 2, Flow control = Xon/Xoff, and then select OK.



6. Save the configuration by selecting File/Save on the next screen. At this point, you are saving the configuration of HyperTerminal under the file name you selected in step 3. The next time you start HyperTerminal, double click on this file, which will allow you to skip steps 3-6 during future interrogations. When asked if you want to save the connection, click "yes" to save the configuration. You can go to the Hyperterminal folder (start → programs → accessories → communications → Hyperterminal folder) and create a shortcut to your connection for the desktop.

7. From the Transfer menu, select Capture Text.



8. Select a folder and a type a file name for the data you will be transferring. This will be the file name for your data. (**Note**: If you select a file that already exists, HyperTerminal will not overwrite the file, it will simply append the data being downloaded to that file.) Select Start.

Steps for collecting LI-1000 data using HyperTerminal

1. Turn on the LI-1000 and select the OUT button.

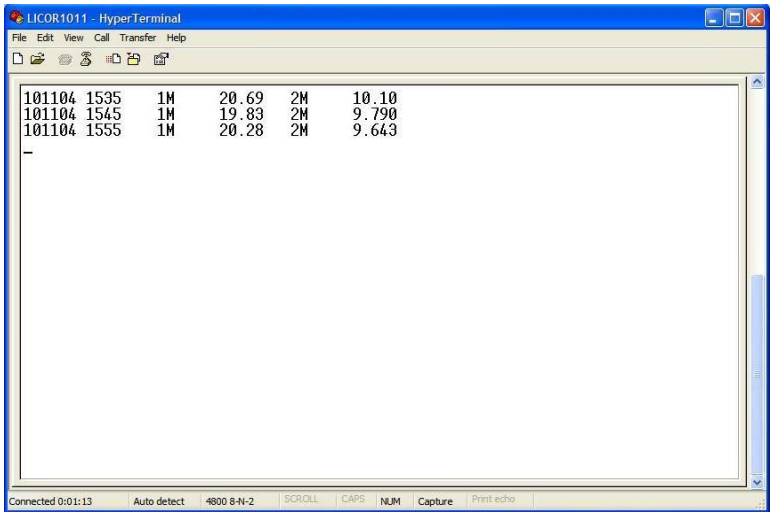
2. Change the baud rate to Baud = 48 (for 4800) by using the up and down arrows and select ENTER.

3. Set the form to Form = H (for horizontal) by using the up and down arrows and select ENTER.

4. Set the len (for length) to len=80 by directly entering the numbers from the keypad and select ENTER.

5. Set the dump all to YES or NO (dependent upon your requirements) by using the up and down arrows and select ENTER. (Selecting NO will prompt you to enter the year, month, date, hour, and minute for starting and stopping your data dump.)

6. Your data will begin to dump to the HyperTerminal program. If you have, for example, two months of data stored in your LI-1000 and choose only to dump the last month, you may have a blank screen for a minute. The LI-1000 will scroll through all readings until it reaches the date marker you set. Upon reaching the date you set for the dump, you will see the data dump to the HyperTerminal screen.



7. To exit the program, choose Call/Disconnect or click on the Disconnect icon on the toolbar, and then exit the program.

Tattle Term Instructions for use of Tattle Term instead of Hyperterminal

1. Open Tattle Term
2. Select Com 1, Click OK, click OK again

3. Select 19200 8N1 in bottom left corner by clicking once
4. Change baud rate to 4800 and stop bits to 2. (Everything else should read Data bits: 8, Parity: none, Com Port: COM1)
5. Select transfer. Click on ASCII and select receive. Enter a filename and save.
6. ON LICOR 1000: press OUT key, baud=48 then ENTER, form=H then ENTER, len=80 then ENTER, dump all=YES then ENTER.
7. Wait until all bytes are transferred.
8. Close Tattle Term and open WordPad (or Notepad).
9. Open file saved in Step 5 through WordPad. (Will be an All Files type)
10. Save this file as a WordPad document.
11. Open Excel. Open file created in WordPad (step 9) through Excel. (All Files)
12. Click NEXT twice, click FINISH.
13. Copy and paste data into Master Spreadsheet.

Underwater Profile PAR and Attenuation (Using LI-1000)

General Discussion

Materials
Licor LI-1000 Data logger with 50 m cable
Licor LI-193SA spherical quantum sensor
Licor LI-190SA quantum sensor
Leveling fixture
Lowering Frame
Tarpaulin (opaque)

Procedure
1. Configure the LI-1000 to record light data on channels 1 (underwater sensor) and 2 (ambient sensor); enter the appropriate sensor multiplier for each channel (See LI-1000 Manual).

2. Configure the LI-1000 to provide instantaneous point values:

CFG	mode is INST
range	A (autorange)
multiplier	**Correct Multiplier on PAR sensor**
label	UM (μmol photons s^{-1} m^{-2})
ave	1

3. Collect the PAR profile data in a covered incubation hole (use a tarp). Situate the incident surface PAR sensor nearby on a level surface, and lower the wet sensor frame into the water. Collect PAR data on 0.5 m increments beginning at just under the surface of the water in the ice hole and continue until the underwater PAR = 0. Once the underwater sensor stabilizes, record both the underwater and incident PAR values.

Note: Always record the time of day and ambient weather conditions (i.e., cloud cover, sunshine, etc.) when profile is collected.

Biospherical Profiling Natural Fluorescence (Original Instrument using DOS software – The instrument broke during the 0708 season, and a new PNF was purchased before the 0910 season.)

General Discussion

The PNF-300 is an integrated optical system specifically designed to measure natural fluorescence - the fluorescence from the phytoplankton community stimulated by available sunlight. Research has shown that natural fluorescence is correlated to ^{14}C photosynthetic rates and chlorophyll concentrations. Unlike strobe fluorometers, a natural fluorometer measures fluorescence emitted under the ambient light conditions which is driving *in situ* photosynthesis.

Materials

Biospherical PNF-300:

- Underwater PNF Instrument with cable
- Surface PAR sensor with cable
- Deck box with AC/DC charger
- Computer cable
- Instruction manual (bring copy from MSU)

PC labtop computer

Procedure

Instrument Calibration

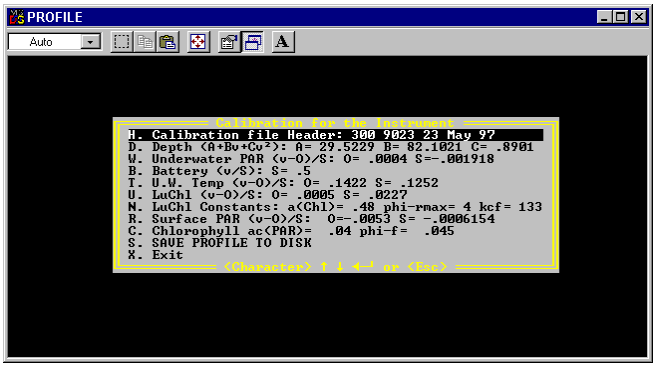
1. Install the PNF-300 software onto the PC laptop. You will need the PROFILE.EXE, PROGRAPH.EXE and the CALIBR8.G30 files. A new folder should be made for the season with these files copied into it. (See Appendix C of the PNF Manual)

2. The PNF 300 should be calibrated every 2 years and a calibration sheet should be supplied. Set the configuration parameters and calibration constants for the data acquisition software. Open the PROFILE.EXE program and press ESC to change configuration. This will bring up the Instrument Parameter Window.

Use the arrow keys to highlight configuration parameters and press ENTER to set the correct values as illustrated above.

3. Highlight "Change Calibration Constants" and press ENTER. This will bring you to the Instrument Calibration Window. Change the calibration constants according to the calibration certificate supplied. **Note**: The calibration constants illustrated below are from a 1997 calibration certificate and will be different from current constants. Save the parameters to disk in both the "Calibration Constants" and the "Instrument Parameter" windows and exit the program. A new calibration file will be written to the folder (CALIBR8.cal). Next time you open the PROFILE.exe program from this folder, these constants will be used. The program is ready for data acquisition.

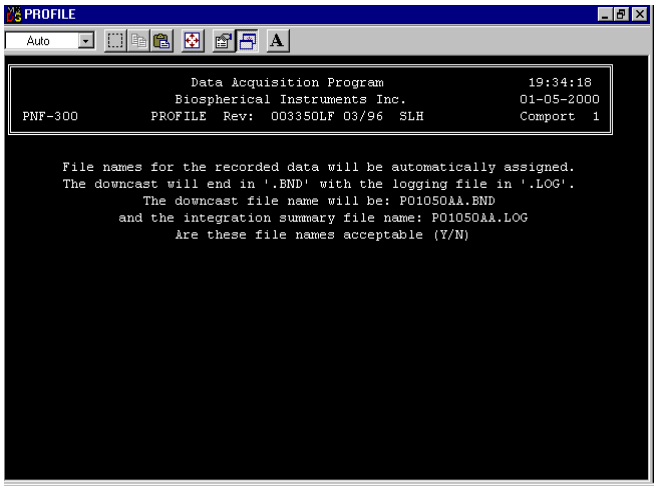
NOTE: Check the serial number on the surface sensor unit to ensure it matches the serial number on the calibration certificate. There is more than one surface sensor in Crary Lab, but only one is sent out each year for calibration with the PNF.



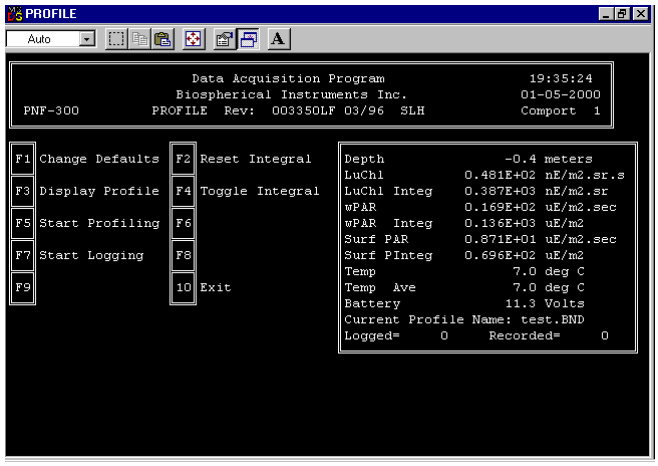
Data Collection

1. The PNF is always cast in an outside hole to minimize shading effects (i.e., Weatherport shading). The sampling hole must be covered with a tarp to minimize direct sunlight entering the hole.

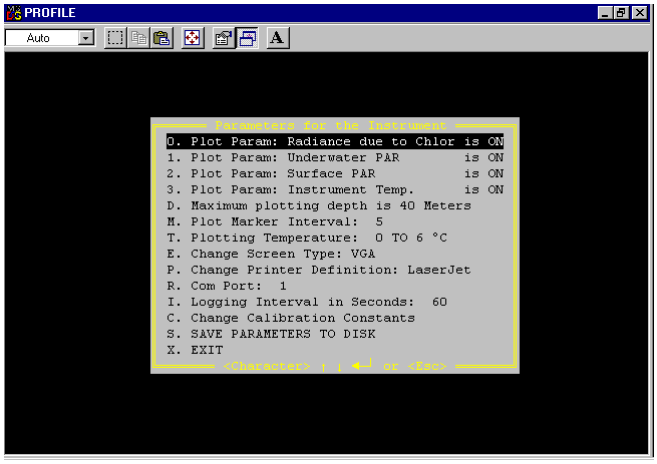
2. Connect the surface PAR sensor and PNF to the deck box, connect the deck box to the computer, and position the cable so the PNF can be easily deployed. The surface PAR sensor should be placed away from the hole to eliminate possible shading. Turn on the deck box and computer. Launch the PROFILE program (shown below) which will initialize the PNF, then enter the appropriate filename when prompted.



3. Position the PNF over the center of the hole and lower the PNF until the downwelling PAR sensor is covered by water. Allow the PNF to cool and the pressure transducer to stabilize. In the PROFILE program select F5 (Start profile) to begin recording data (shown below). Slowly lower (0.5 m sec^{-1}) the PNF to the desired depth. Continue recording data on the upcast and pause at the surface for 10 seconds for reference. Select F6 (Stop profile). Perform a second downward cast (if desired) and rinse the PNF.



5. View the data to ensure the instrument was working properly. Following the cast, a .bnd and a .txt file will be generated. Launch the PROGRAPH.EXE program and select the appropriate filename (only .bnd files will be displayed. These files must be in the same folder as the PROFILE.EXE program for the program to read them). Select “plot” to examine the data. Select “convert to CSV” to convert the .bnd file to a .csv file. This is the file you will use to see the data. Exit the program.


PROFILE
Auto
0. Plot Param: Radiance due to Chlor is ON
1. Plot Param: Underwater PAR is ON
2. Plot Param: Surface PAR is ON
3. Plot Param: Instrument Temp. is ON
D. Maximum plotting depth is 40 Meters
M. Plot Marker Interval: 5
T. Plotting Temperature: 0 TO 6 °C
E. Change Screen Type: VGA
P. Change Printer Definition: LaserJet
R. Com Port: 1
I. Logging Interval in Seconds: 60
C. Change Calibration Constants
S. SAVE PARAMETERS TO DISK
X. EXIT

Seabird 25 Conductivity, Temperature and Depth Instrument
(USING SEASAVE WIN32 V5.39)

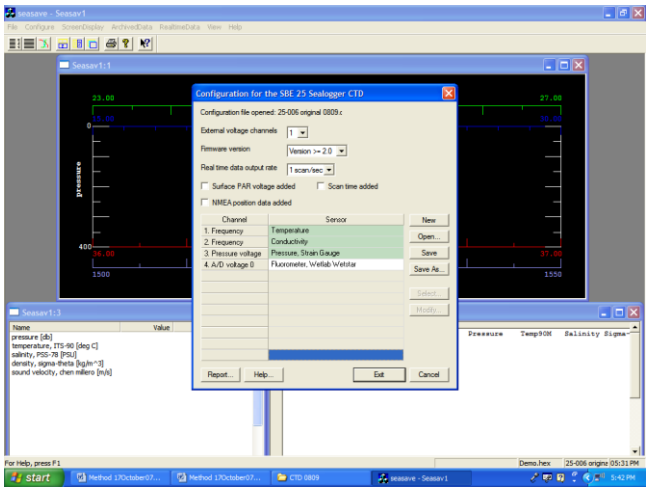
General Discussion

Materials
Seabird 25 CTD (80 m)
PC laptop computer
1% Triton-X solution
60 ml syringe and tubing to connect to the pump outlet

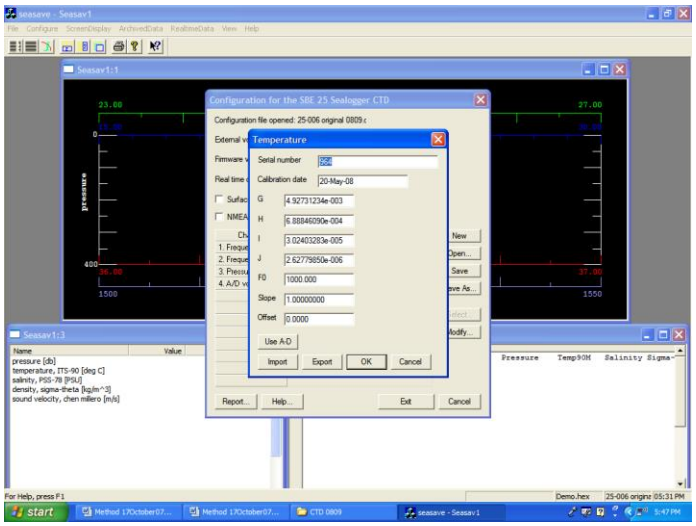
Procedure
Instrument Calibration (SeaSave)
1. Install the current version of Windows Seasoft data acquisition software onto the laptop computer. **SeaSave** is used to view and change .con file data, **SeaTerm** is used to communicate with the CTD, do the casts, and download the data, **SeaSave** is used to view the data, and **SBE Data Processing** is used to process the data (convert .hex files).

2. Configure the data acquisition software based on the current calibration constants provided by Sea-Bird Electronics. Every year the manufacturer sends a paper copy of the calibration values, and an electronic copy of the .con file containing these calibration values. Save the current .con file to the computer from the CD and view it in SeaSave to check the values. In SeaSave, click on "configure" → "new style instrument configuration" then "select instrument configuration" and choose the appropriate .con file. Go to "modify selected instrument configuration." If you don't have a .con file, you can modify an existing file with the values from the paper copy. Enter the values as illustrated below:

- Enter "> than highest" for firmware (Gary Morast from SeaBird said that the Firmware version is important to have correct. In 0708 it was 4.02)
- "8 scans per second" for Data Output Rate (this is user configurable: When our CTD come back from the manufacturer, it is set to store data at 1 scan per second; we will change this to 8 scans per second using SeaTerm (see below)).

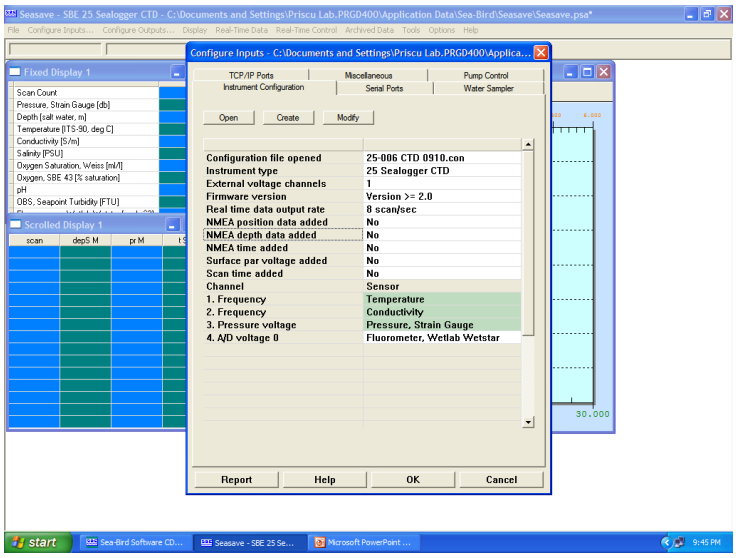


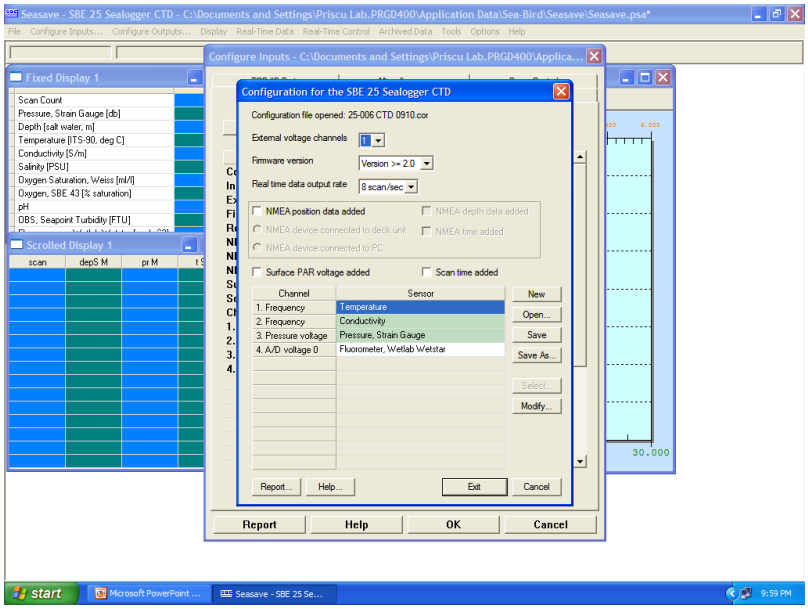
Check the Temperature, Conductivity (Set the Cell Constant = 2000, and the Series Resistance = 300), Pressure and Fluorometer values, or enter them from the paper copies of the calibration reports if you are not using the current .con file. If you have selected the current .con file, these values should match the values in the paper copy. Below are the 0809 calibration coefficients for the Temperature Sensor.



Save the configuration file. The .con file is used for data processing (conversion of .hex files) with SBE Data Processing, and for viewing data in SeaSave. SeaSave and SBE Data Processing will not interpret the data correctly without the correct .con file.

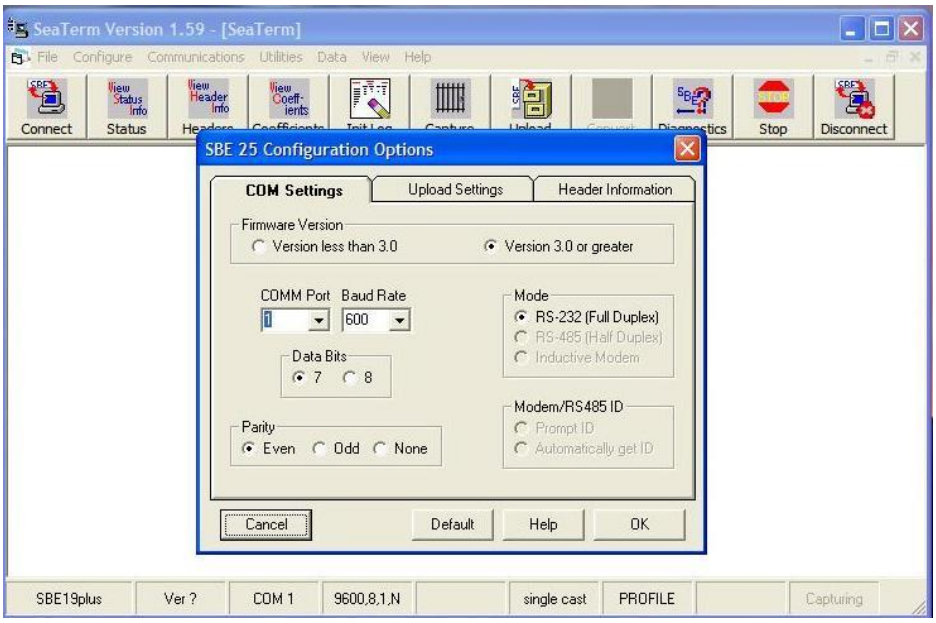
If using the new SeaSave Version 7, go to “Configure Inputs” → “Instrument Configuration” and then “Modify” to check and change any of the values as described above.





Instrument Setup (SeaTerm)

2. Before using SeaTerm to communicate with the CTD or upload data from the CTD, the program must have information about the CTD hardware configuration and about the computer. To set these parameters, open the SeaTerm program, go to “configure” and click on “SBE 25” (that is the name of our CTD).



Information about the CTD's configuration came with the original instrument purchase:

COM settings (must match CTDs configuration sheet):

a. Firmware: > 3.0 (updated to 4.02 in 0708) **(must be correct according to Gary Morast of SeaBird)**
b. Comm port – 1
c. Baud Rate – 600 (the program cycles through the baud rates until it finds the one it can use to connect. You can either put 600 and let it find the right one, or put the one it uses (usually 4800) to start out with).
d. Data Bits – 7
e. Parity – even

Upload settings:

e. Data Upload Baud Rate – 9600
f. Upload Data: 1 cast (set as desired)
g. SBE 3 - Temperature Sensor Serial Number: 0964 **(must match .con file)**
h. SBE 4 - Conductivity Sensor Serial Number: 2396w **(must match .con file)**

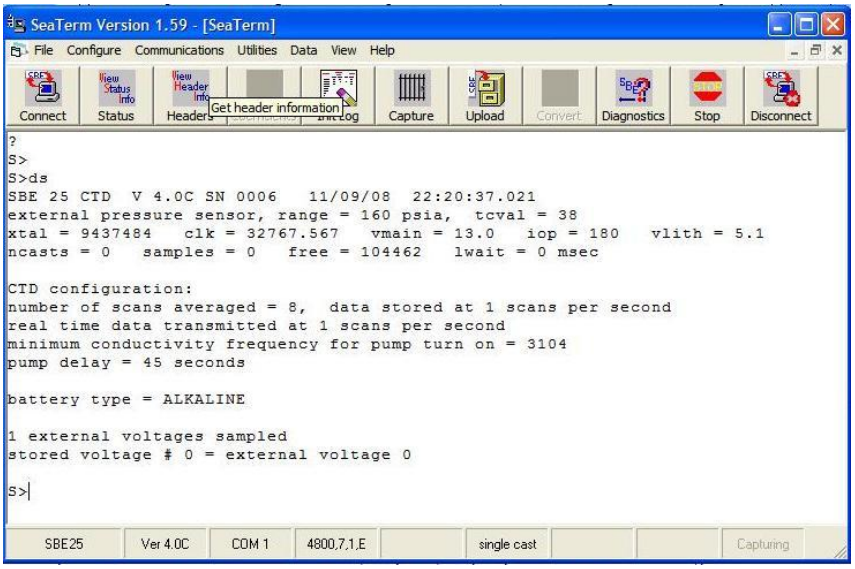
Header Information (set as desired):

a. Header: Prompt for Header Information

When done, click OK and SeaTerm will save the settings in a SEATERM.ini file. SEATERM will not upload data correctly without a properly configured SEATERM.ini file. (If the program saves the settings, it has created the .ini file in the root directory; you don't actually have to do anything with the .ini file).

Click "Connect" to communicate with the CTD

Click “Status” to get information about the CTD.



When the CTD comes back from the factory every year, it is set to turn on at a salinity meant for using it in seawater. This parameter is called the “minimum conductivity frequency for pump turn on.” Since we are using it in the lakes, we need to re-set this value so that the CTD will turn on in relatively freshwater. After you have established communications (above), check the minimum conductivity frequency for pump turn on in the CTD configuration list that appears. Generally it will be ~ 3000 Hz from the factory. To set the new pump start frequency:

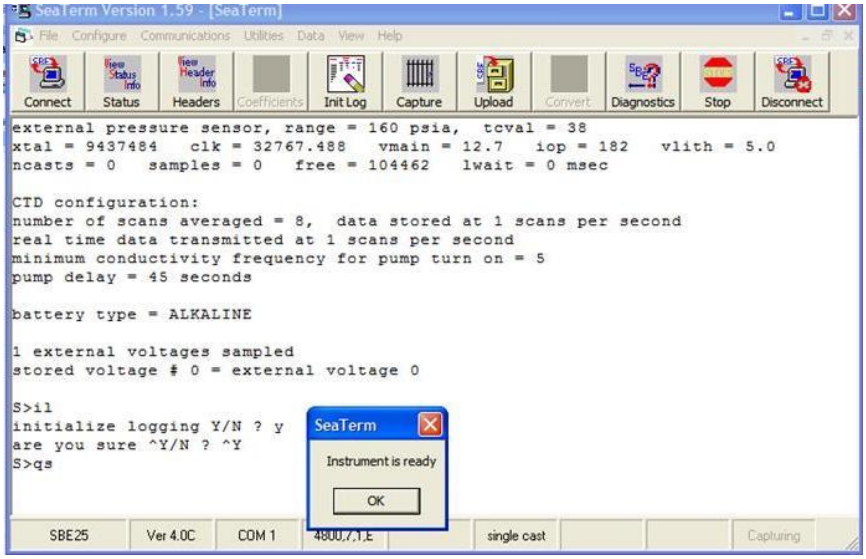
At the s> or #s> prompt, type cc
type Y when it asks Y/N
type CTRL Y when it asks CTRL Y/N
Press enter to keep the rest of the values as they are, but change the pump start frequency to 5.

When the CTD comes back from the factory every year, it is set to a Real Time Data Output (or Transmission) Rate and Data Storage Rate of 1 scan per second. We need to change these to 8 scans per second. (The con file generally has the Real Time Data Output Rate set to 8 scans per second. This will have been checked in SeaSave already). After you have established communications, check the Data Storage Rate and the Real Time Data Transmission Rate in the CTD configuration list that appears (see above). Generally they will have been set to 1 at the factory. To set the new rates:

At the s> prompt, type cc
type Y when it asks Y/N
type CTRL Y when it asks CTRL Y/N
Press enter to keep the rest of the values as they are, but change the Internal Data Storage Rate and the Real Time Data Output Rate to 8.

Data Collection (SeaTerm)

2. Before sampling, the SBE 25 CTD must be initialized to clear memory modules. Connect the SBE CTD to the computer and launch the SeaTerm communication program. Press "Connect" to communicate with the CTD, and "Status" to get information about the CTD. Following recognition, press "Init Log" to initialize logging, answer YES to the questions.



Disconnect the cable from the CTD and replace the protective plug. The SBE 25 is now ready for deployment. "qs" refers to quiet or quiescient state. The CTD must be in this state for 2 minutes before the pump turn on switch will work. You can command "qs" in SeaTerm (it automatically did this above), or disconnect the CTD and be sure to wait 2 minutes until turning on the pump switch.

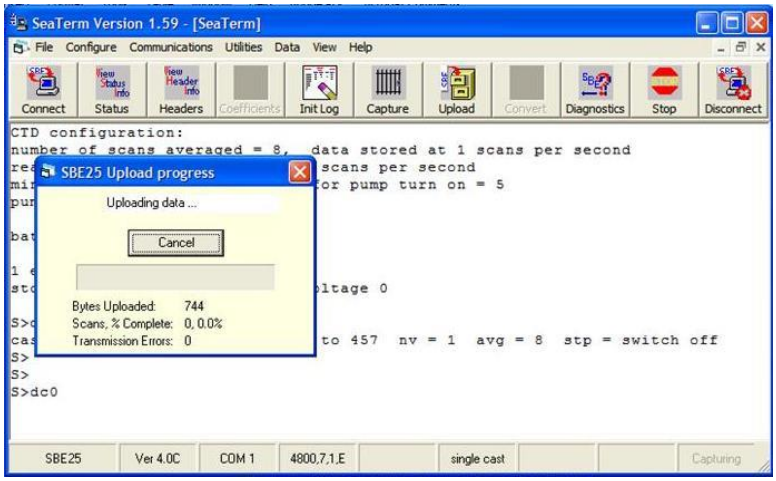
2. Typically the SBE 25 CTD is cast in the Weatherport sampling hole, because it does not measure natural fluorescence or PAR which is influenced by the shading effect from the Weatherport. Remove all of the ice from the hole, **Do Not** allow ice crystals to touch the probes. Attach the CTD frame to the winch cable. Remove the red cap. Use a 60 ml syringe to flush the pumping system and sensors with a 1% Triton-X solution.

3. Position the CTD over the center of the hole and turn the unit on. Immediately lower the CTD into the water up to the O ring where the frame is attached to the cable. Allow the CTD to thoroughly flush water through the system (>60 sec – it takes 45 second for the pump to turn on once it is put in the water). The CTD may have to be raised out of the water enough to see that the pump is working. The pump will only turn on at the salinity you entered in step 2, Instrument Set-up. Once you ensure that the pump is working, lower the CTD to the O ring again, and let it sit for a minute. This depth is 0.8m from the pressure sensor, and will be used to correct the pressure sensor to the correct depth during data processing. This is very important as the pressure sensor is not always accurate!!

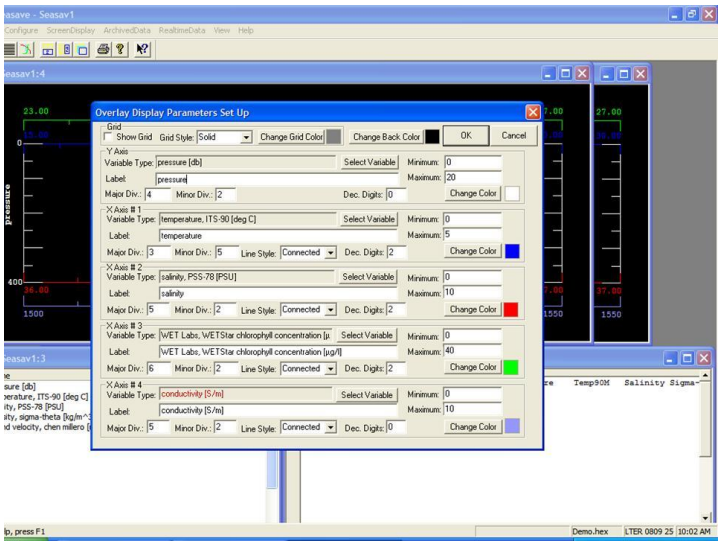
4. Slowly lower the CTD (1 m sec^{-1}) to the desired depth ~2 m from the bottom of the lake **(Do Not** allow the CTD to hit the bottom of the lake), stop for 10 seconds, then slowly retrieve at the same rate. Remove the CTD from the water and immediately turn the instrument off. **Note**: The CTD must be turned off to save each cast. Following data collection, turn the instrument on and lower to 1 m to thoroughly rinse the instrument, remove and place in carrier. Flush the pumping system with DI water after use.

Downloading (SeaTerm)

7. Connect the SBE 25 CTD to the computer and launch the SeaTerm program. Once communications are established press "Upload" to Upload data. **Note**: The software may be configured to dump specific casts or all, and to include data headers. Name the file accordingly (i.e., Lake code, date), and save to the desired directory. Every time the pump is turned on and off there is a new cast saved. Therefore, when downloading the data the first cast is 0, second is 1, etc. The multiple casts can be downloaded, but they must be specified when you "upload" the data in SeaTerm.



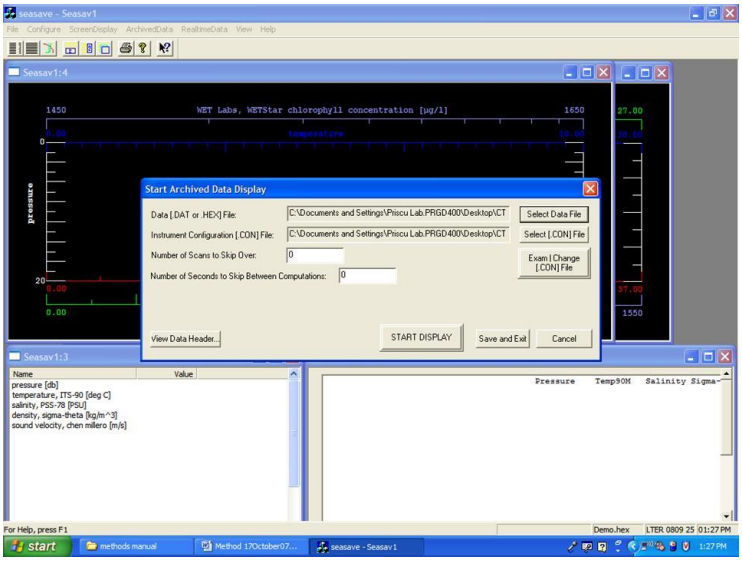
2. View the data to make sure the CTD was operating properly using SeaSave.
    1. Create a DSO (display set-up file) file (one for each lake) or select the appropriate DSO file if it has already been created:
        a. Select "Screen Display" → "add new display window" → "overlay display" (this is the DSO screen we usually work in).
        b. Select "Screen Display" → "edit selected display window" → "Modify display parameters." Enter the range of parameters for each lake (if you need to change a parameter, press the "select variable" button).

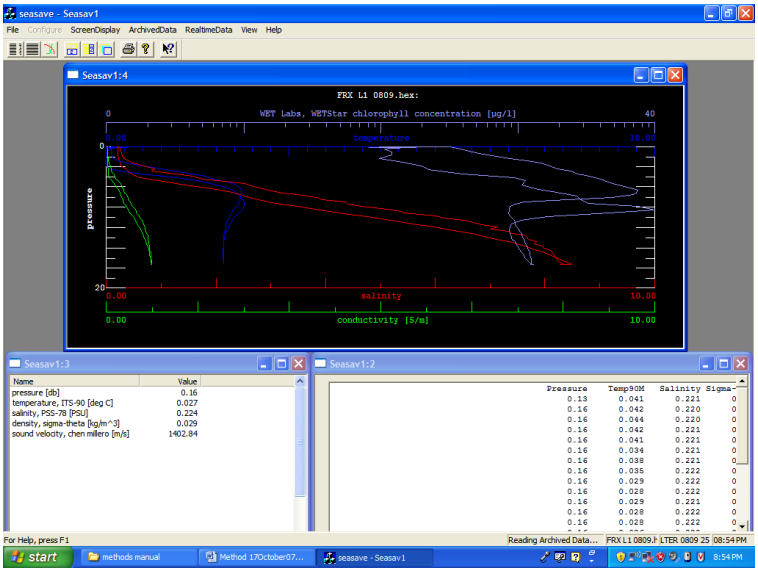


Save the DSO file in a desired location. You can create a file for each lake at this time if you prefer.

c. If you want to select a DSO file you have already created, Select "Screen Display" → "edit selected display window" → "select DSO file"

2. To display the data from your cast, go to "Archived Data" → "Start." Select data file, select CON file. Click on "Start display."



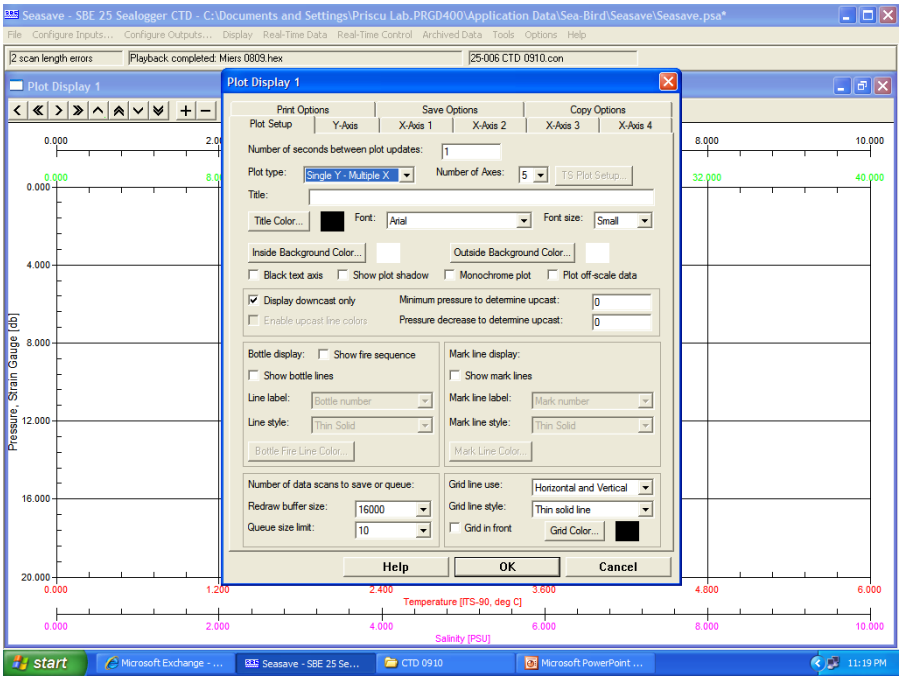


If using the new SeaSave Version 7, you can view a plot as described above, or view a readout of the data.

To view a readout of the data, go to "Display" → "Add new fixed display window." Then go to "Archived Data → Start," select data file and .con file, change number of scans to skip over at start to ZERO (or however many scans you want to ship over), and press start.

To view a plot of the data, go to "Display → "Add new plot display window."

Under the "Plot Setup" tab make the following selections:
Plot Type: Single Y – Multiple X
Number of Axes = 5



Under the "Y-Axis" tab make the following selections:

Set up the y-axis with Pressure (db)

Under the "X-Axis" tabs make the following selections:

Set up the x-axes with:

Temp (ITS-90, °C)
Salinity (PSU)
Fluorescence (WetLab Wetstar (mg/m3)
Conductivity (S/m)

Set up an appropriate range for each parameter for the lake which you are plotting. Make any changes to color, etc desired.

To save graph set-up, close set-up window, right click on graph, and "Export Display Settings (.dsa file)."

To re-enter plot set-up screen, open graph and click on "Display → Modify," or right click on graph and click on Modify.

To open a dsa file that was already set up, Display → "Import Display Settings (.dsa file).

To draw the graph, go to "Archived Data → Start," select data file and .con file, change number of scans to skip over at start to ZERO (or however many scans you want to ship over), and press start.

Note: In past years, problems with data acquisition have occurred when cable connections allowed water to infiltrate and corrode the connections. If data appears to be chaotic, remove all cable connections and thoroughly dry the connections. Lube the connectors with stopcock grease and securely tighten the cables.