Arctic LTER Streams Protocol
Field and Lab Methods
Toolik Field Station and
RESL at the University of Vermont

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I. General Toolik Field Sampling Info

Sampling of many different things occurs throughout the summer for the LTER Streams group. The Kuparuk River and Oksrukuyik Creek are the main sampling locations for the LTER, but other sites may be added in different years. These protocols are specific to the long-term sampling of the Kuparuk River near the Haul Road and Oksrukuyik Creek near the Haul Road, and Imnavait Creek surrounding the access road and thermokarst. Additional streams that are sampled are not discussed in detail because the streams change regularly and sampling protocols may differ slightly, however, all chemistry methods remain the same, or allow for overlap of analyses. Other types of streams that have been studied have included streams that are impacted by thermokarst, tributaries of a stream network, and spring, glacier, and mountain streams. The Kuparuk has had phosphorus added to it every year since 1983, Oksrukuyik had phosphorus and nitrogen added 1991-96. Reference, recovery, and fertilized reaches are included in the sampling regime. Each stream is sampled three times during the summer for nutrients and benthic chlorophyll (rock scrubs). Regular discharge measurements are taken on both streams. Arctic grayling are caught and released, both adults and young of the year. Macroinvertebrates, both with Surber samplers and drift nets, are collected at least twice from the Kuparuk, less frequently from Oksrukuyik. Moss point transects are done on the Kuparuk twice during the field season.

A. Wet Lab

The Wet Lab at Toolik is a highly used lab space – many people are often coming and going. It is important to follow some rules so that everything runs smoothly – including both personal safety and good science.

1. **Some rules**
   - **a.** Outside shoes are not allowed
   - **b.** Designated, close toed lab shoes are required
   - **c.** Stay out of the RAD area
   - **d.** Follow the Nutrient RA's rules and guidelines
   - **e.** Sign up for analyses
2. **Acid washing**
   - **a.** Two acid baths are on the front porch – one for NH4 equipment and one for phosphorus/anything else – only use the NH4 bath if the Nutrient RA says it is ok
   - **b.** rinse everything that is going in the bath with DI 3x
   - **c.** remove all tape!!! It makes everyone happier – when tape has been acid washed it becomes very gooey, just making it more difficult to remove the next time that you try.
   - **d.** using long rubber gloves, rubber apron, and goggles put lab ware into the bath
   - **e.** let soak for ~1hour

- only soak filter cartridges with metal for ~5minutes!

f. remove the items from the bath – wearing the gloves/apron/goggles
g. rinse with DI 3X – pour the waste DI/acid into a bucket to be disposed of in the acid waste (or to mop the floor with)
h. let dry
i. put items away

B. Field Sites

1. ***Kuparuk River***
   a. The original, 1983 phosphorus dripper was located at what is called 0.0k – all other stations are named as a distance from that location. Positive numbers are downstream; negative numbers are upstream. Stations downstream of the dripper were in the fertilized reach, upstream are reference stations.
   b. The phosphorus dripper was moved downstream in 1985 to the location called 0.56k. It remained at that location until 1995.
   c. In 1996 the dripper was moved downstream again to 1.4k and remained there until 2010. Stations upstream of the dripper were in a recovery reach. Downstream of the current dripper location is fertilized and upstream of the original dripper is still reference.
   d. In 2011, a second dripper was added at the 0.0k location. Phosphoric acid is added at a rate of 2.4ml/min. This is in addition to the dripper which has been at 1.4k since 1996, dripping at 1.2ml/min. The area between 0.0k and 1.4k has become known as the re-fertilized zone. This zone is broken up into two "Re-fert" zones, divided at the location of the dripper at 0.56k from 1985-1995.
   e. Stations for long-term data remain the same from year to year, but some stations are sampled for different things, depending on habitat characteristics. Most stations that are frequented are riffles.

Station Name	***Reach type***	***Samples Collected***
-0.7k	*Reference*	*YOY*
-0.47k	*Reference*	*YOY*
-0.377k	*Reference*	*Nutrients, rock scrubs, macroinvertebrates, moss point transect*
-0.177k	*Reference*	*Nutrients, rock scrubs, macroinvertebrates, moss point transect*
0.0k	*Reference*	*Original 1983 phosphorus dripper, YOY, moss point transect, macroinvertebrates*
0.3k	*Reference*	*YOY, moss point transect*

0.5k	*Reference*	*High flow discharge measurement, fish weir*
0.56k	*Reference*	*Nutrients, rock scrubs, moss point transect*
0.59k	*Fertilized/Recovery*	*1985-1995 phosphorus dripper*
0.6k	*Fertilized/Recovery*	*Discharge measurement*
0.74k	*Fertilized/Recovery*	*Nutrients and rock scrubs*
0.85k	*Fertilized/Recovery*	*YOY*
1.0k	*Fertilized/Recovery*	*Nutrients, rock scrubs, YOY, and moss point transect*
1.15k	*Fertilized/Recovery*	*Moss point transect*
1.2k	*Fertilized/Recovery*	*Moss point transect*
1.39k	*Fertilized/Recovery*	*Nutrients and rock scrubs*
1.4k	*Fertilized*	*1996-2010 phosphorus dripper*
1.8k	*Fertilized*	*Nutrients, rock scrubs, YOY, moss point transects*
2.0k	*Fertilized*	*Nutrients, rock scrubs, moss point transects, macroinvertebrates*
2.5k	*Fertilized*	*Nutrients, rock scrubs, macroinvertebrates*
3.0k	*Fertilized*	*Nutrients, rock scrubs, YOY, moss point transects, macroinvertebrates*
4.0k	*Fertilized*	*Nutrients, rock scrubs*
4.1k	*Fertilized*	*Moss point transects*
Hershey Creek	*Tributary*	*Nutrients*

2. *Oksrukuyik Creek*

a. Oksrukuyik Creek was sampled beginning in 1989 to study baseline conditions. Fertilization using phosphoric acid was begun in 1991, increasing SRP by 0.32 uM. Phosphorus addition continued through 1996.
b. Ammonium sulfate was added 1993-1996, increasing N by 7.1 uM.
c. Recovery from nutrient additions was begun in 1997. No further additions have been made since then.
d. Sampling in the stream occurs primarily at the reference reach stations to continue baseline monitoring.
e. Discharge was formerly measured downstream of the Haul Road near the USGS stilling well, however, in 2008 the USGS equipment was removed due to impending road construction. The discharge measurement site was moved upstream of the road ~100m.

Station Name	***Reach Type***	***Samples Collected***
-0.7k	Reference	Nutrients, rock scrubs, YOY
-0.3k	Reference	Nutrients, rock scrubs, YOY
-0.2k	Reference	YOY
-0.1k	Reference	Nutrients, rock scrubs, YOY
0.227k	Fertilized/Recovery	Nutrients, rock scrubs
0.45k	Fertilized/Recovery	YOY
0.482k	Fertilized/Recovery	Nutrients, rock scrubs
0.85k	Fertilized/Recovery	YOY
1.056k	Fertilized/Recovery	Nutrients, rock scrubs
1.370k	Fertilized/Recovery	Nutrients, rock scrubs, YOY
1.700k	Fertilized/Recovery	Nutrients, rock scrubs
2.497k	Fertilized/Recovery	Nutrients, rock scrubs

3. *Imnavait Creek*

a. Imnavait Creek is sampled at least twice during each summer by the streams group in the thermokarst-impacted reach.
b. Nutrient samples and total suspended sediments are collected at each station.
c. Filtering is often done in the lab because more particulate matter is in the water, making syringe filtering in the field difficult.
d. It is important to collect from areas of running water, which can sometimes be difficult in dry years

Station Name	***Station Type***	***Station Description/Location***
A	Reference	Upstream of the boardwalk
AA	Reference	Inlet to 2nd pool upstream of the culvert
B	Impacted	Outlet of pool ~50m below access road
C	Impacted	~250m downstream of road (wooden stake and rebar mark the station)
D	Impacted	50m upstream of the black diamond sign
E	Downstream of impacted area	~50m upstream of Haul Road – inlet to the large pool

C. Nutrients

On a nutrients day water samples are collected for NH_4 and SRP to be run in Wet Lab at Toolik. Sestonic chlorophyll *a* samples are also collected and run at Toolik. Other samples are collected, but preserved for analyses at the RESL or other home institutions, including NO_3, TDP, TDN/DOC, PP, PNPC, anions, cations, and alkalinity. In addition to water sample collection, pH, temperature, and conductivity are also measured.

1. Pre-field prep – what to take

Pack everything the day before going into the field – it makes everyone's life easier

a. Field book and pencils
b. amber 1 L bottle for chlorophyll a samples
c. Station bags containing:

Item	**Analyses**	**Total needed per station**
125 mL HDPE amber	NH4/SRP (reuse, must be acid washed/DI rinsed between sampling) (1/regular, 2/rep station)	1/regular station, 2/rep station
60 mL LDPE	Nitrate(1/regular, 2/rep station)	1/regular station, 2/rep station
60 mL HDPE square	Cations (only done at rep station)	1/rep station, 0 per regular
60 mL HDPE narrow mouth	TDN/DOC (1/regular, 2/rep station), TDP (1/regular, 2/rep station), Alk (only done at rep stations)	2/regular, 5/rep
30 mL HDPE	Anions (only done at rep stations)	1/rep station, 0/regular
47mm Petri dish	PP, PCN (1 each/station)	2
25 mm filter cartridges	PP, PCN (1 each/station)	2
Whatman 45um Puradisc filters	Cations (only done at rep station)	1/rep station
10ml Falcon Tube	Chl a (1/station)	1

d. 25 mm filter cartridges can be pre-loaded with ashed 25mm GF/F **the morning of field collection** (to pack cartridges always use forceps, never touch the filter! Place in the center of the metal disc, place the O-ring on top of the filter, screw the top part of the cartridge on tightly, but don't over-tighten or the filter might break).

e. Syringe box – at least 2 140cc clean syringes, filter forceps, sharpie
f. Extras bag - Forceps, extra filters, an extra filter holder and O-ring, labeling tape and syringe, sharpie, batteries
g. Calibrated pH/conductivity meter and probes
- set up and calibration - use the pH buffers in Wet Lab to calibrate the Hach pH probe before use each day
  - use pH 7 and 10
  - rinse with DI between each buffer
  - make sure the electrode has gel – when it is cold this can get too thick so you might need to try to shake it or click more gel if using the electrode with gel replacement capsules
  - follow the instructions in the manual for calibration
  - pour waste into the pH buffer waste container
- make sure there is extra buffer in the case so that field calibration can be done if necessary

2. Field methods – what to do

a. Nutrients, sestonic particulates, and ions

- At each station, go to the center of the stream and rinse the syringe 3x with stream water.
- Fill the syringe with stream water, mount the 25mm filter cassette onto the syringe and push 5-10 ml of water through the filter to rinse – also check to make sure no water is coming out of the sides of the cartridge – the filter might not be seated correctly if you see water. When filtering always point the syringe towards the ground. Fill bottles with filtered water, **rinsing each sample bottle 2x with filtered stream water** before filling. Keep track of the volume filtered.
- After the last water has gone through the filter push a small amount of air through the filter by removing the cartridge from the syringe (or turning off the stop-cock) pulling a small amount of air in the syringe and then pushing it through the filter (if you don't remove the cartridge you will pull air through, possibly breaking the filter), remove the filter with the forceps and place it in a petri dish labeled for particulates (see below, #8).
  - **Cations** – use a .45um filter for the cation sample – sometimes it can be very difficult to use these filters so a caulk gun can be helpful, but be careful to not break the syringe! The square 60mL bottles are used for cations so that you remember to use the other filter – so if you run out of squares, it is ok to just use a regular 60ml.
- **Record the volume of water filtered through the filter directly on the labeled petri dish with a sharpie**.

- **Avoid making any physical contact with the filtered water!** Your skin can contaminate the water for certain analyses. Keep mosquitoes and unfiltered stream water out of the sample bottles.

b. Sestonic Particulates (PN, PC, PP)

- After each filter has had at least 500 mL (for Kup and Oks, you may not be able to filter 500 mL on other streams) of water run through it, remove the filter with the forceps and place it into one of the Petri dishes (it doesn't matter which one). The dishes should be pre-labeled with stream, station date, and either PN/PC (particulate nitrogen and particulate carbon), or PP (particulate phosphorus). Place the other filter into the second pre-labeled Petri dish. **IMPORTANT: record the volume of water filtered through the filter directly on the labeled Petri dish with a sharpie!!**
- Remember to dry field filter blanks for each type of particulate sample (1/transect). The blanks should be from the same box as the sample filters and should be dried on the same day.

c. Sestonic chlorophyll *a* concentration

- At each station, rinse the syringe 3x with stream water. Take the samples from the center of the river.
- Fill the syringe with stream water, then attach the 47mm filter cassette (with filter) to the syringe and push water through the filter. Repeat this step until 500 mL of stream water has passed through the filter.
- Push a small amount of air through the filter cassette using the syringe (this pushes the remaining ~5mL of water through the filter and prevents sample loss in the transfer)
- Remove the filter from the cassette and place in a falcon tube, and pace this tube in a amber dark bottle so that the Chlorophyll levels don't change.

d. pH/conductivity

- put the electrode directly in the stream, but in an area that is not too turbulent
- if the pH is reading strangely try recalibrating in the field with buffer that is kept in the case
  - o if still having problems, try reading the pH in a sample bottle instead of directly in the water
  - o check the amount of gel on the electrode
  - o if all else fails, read the samples back in the lab with a different pH probe

3. Return from the field – what to do with all of the samples you just collected

a. Rinse syringes and filter cartridges with DI 3X – let dry in clean bin (if you don't rinse these before they dry you must acid wash them)
- Remember NOT to acid wash the chlorophyll syringes or filter cassettes between uses; acid rapidly degrades chlorophyll, so traces of acid from the acid bath could affect the chlorophyll in the samples.

b. Open pH/conductivity meter box to let air dry

C. Some samples are run immediately, and others are preserved for later analysis. The following chart tells you where everything goes from a nutrients day.

Sample	**What happens to it**
NH4/SRP	Run samples on day collected, if can't be run w/in 24hrs, freeze
TDP	Acidify w/ 100uL 6N HCl, refrigerate
TDN/DOC	Acidify w/ 100uL 6N HCl, refrigerate
Cations	Acidify w/ 100uL 6N HCl, refrigerate
Nitrate	Freeze
Alks	Refrigerate
Anions	Refrigerate
Sestonic particulate phosphorus filter	Dry in oven 60^{o}C for 24 hours, then store for shipment
Sestonic particulate carbon and nitrogen filter	Dry in oven 60^{o}C for 24 hours, then store for shipment
Sestonic chlorophyll *a*	Put in designated 15cc centrifuge tube, freeze 1 hour – 1 week, analyze on fluorometer (see methods in lab analysis section)

D. Rock Scrubs

Algae growing on the stream bottom rocks are an important component of primary productivity. Therefore, samples of epilithic algae are scrubbed off of the rocks and their chlorophyll content is measured. Benthic particulate nutrients are measured as well.

1. Pre-field prep – what to take

a. field book and pencil
b. wash basin
c. small steel bristle scrub brush
d. 500-ml wash bottle (whole rock method)
e. 250 mL sample bottles – two for each station, reuse dedicated bottles

f. 2x2 slide holders (2x2 method)
g. 3-5 60 ml bottles or 56 ml centrifuge tubes. (2x2 method)
h. funnel (optional)
i. black garbage bags

2. Field methods – what to do

Whole rock scrubs - The 2 x 2 rock scrubs outlined in the Appendix is a fast method used to determine the chlorophyll α and particulate nutrients in the epilithic algae. Whole rock scrubs are a more thorough method for the same purpose. Once a summer, whole rock scrubs were conducted at the same time as 2 x 2 scrubs to compare the results and efficiencies of the two methods. **Note: Since about 2004, we've been doing whole rock scrubs only and have stopped doing 2x2 since whole rock is the preferred method (Bruce Peterson; personal communication).** The methods below are those of Bruce Peterson (personal communication) and have been modified into outline form.

a. At each station where whole rock scrubs are to be done, rinse the wash basin, graduated cylinder, brush, and squirt bottle 3x with river water.
b. Select several rocks from each station that fit the following criteria (<u>make careful notes of any rocks that do not fit the criteria</u>):

   - no obvious filamentous algae or moss (to avoid overestimates of chl due to filamentous algae or moss)
   - fairly smooth surface (uneven surfaces prevent efficient removal of epilithon)
   - similar size (the rocks should be of appropriate size to form a single layer of rocks that fills the bottom of the wash basin).

c. Remove the rocks from the basin and gently set them aside, right-side up. Place them in shallow water if you are able to keep track of which rocks you chose
d. Fill the 500ml rinse bottle with river water. You will need to use 2-500 ml rinse bottles of water to have a 1L initial volume. <u>All of this water will be needed, so do not spill or waste any.</u>
e. One by one, scrub each rock vigorously and thoroughly with the wire scrub brush. All scrubbate should fall into the basin.
f. Rinse each rock with the water from the wash bottle, making sure that all rinse lands in the basin.
g. After all scrubbing is complete, the basin will contain a slurry of 1 liter (fill the 500ml rinse bottle twice) of water plus all of the scrubbate.
   - Stir up the slurry in the basin so that it is homogenous; then fill the pre-labeled 250-ml bottle with slurry. This is a subsample for laboratory analysis.

- Pour out the remaining slurry. Store the bottle for return to camp.
- Repeat this procedure twice, so that at each of the stations, you scrub a total of two basins of rocks.

3. Return from field – what to do with all the samples you just collected

a. Using forceps place pre-combusted Whatman GF/F 25 mm glass fiber filter onto frit; attach upper reservoir to filter holder.
b. Pipette 5 ml (note if otherwise) from each replicate sample bottle (shake bottle first) into filter reservoir. Turn on vacuum pump, be sure not to allow pressure differential to exceed .3 ATMs, to minimize damage to delicate organisms and don't let filter pull dry (slowly release vacuum as final volume of water is filtered).
c. Place filter into centrifuge tubes (Falcon Tubes) and place in the freezer for at least an hour (no longer than one week). Then add 10ml of chilled 90% acetone (1mg $MgCO_3$/L). Make sure filter is completely immersed in acetone, cap tightly and place in light tight box while processing, then move to cooler with ice for extraction. These filters will be analyzed for Chlorophyll *a* content after a 16-18 hour extraction period. Tubes should be inverted/mixed at least once during extraction, but allow sufficient time (>4hours) for particles to settle before reading.
d. Once you have filtered 5 ml for chlorophyll for each replicate from a station, combine the remaining sample (5 for 2x2's and 2-3 for whole rocks) into a large bottle with lid. Shake the bottle and then filter 5ml (write volume on Petri dish) of this homogenate through 2 more GF/F 25mm filters for PNPC and PP samples.
e. Place these PNPC and PP filters in petri dishes and place in the drying oven for at least 24hrs with the lids ajar. (That's it for the PNPCPP- store in a ziploc bag and ship to RESL).see LTER Streams sampling protocol.
f. Pipette 20 ml of the homogenate into glass scint vials for algal comps. Preserve with 0.1ml 50% glutaraldehyde. Ship to UVM Rubenstein Ecosystem Science Lab..

E. Discharge

1. In each stream, discharge will be measured manually <u>at least 6 times (preferably 8 times) per summer</u> at intervals of approximately one week or following significant changes in stream levels. The purpose of these measurements is to create a water level/discharge curve so that the datalogger water level data can be converted to discharge data.
2. Read and record the river depth on the staff gauge on the side of the stilling well. The units are in hundredths of feet. Record the time of day as well.
3. At the designated station in each stream extend a meter tape, perpendicular to the flow, across the river and secure it on both banks.

The tape should be relatively taut. This will serve as a reference for doing the transect.

4. Measure the width of the stream and divide the width into 20 increments. Write these increments in one column of the notebook.
5. Beginning at one bank, measure and record the depth of the river, then measure and record the current velocity using the Gurley, Marsh-McBirney, or SonTek ADV current meter.

    a. If using the SonTek ADV name the file with the first letter of the river (K for Kuparuk) and the date using DDMMYY format. With the 3D ADV probe attached to the wading rod, point the probe upstream, stand downstream of the probe so that you don't create an eddy. However, if the water is very clear, you may get a poor reading, in which case one person should stand upstream of the probe and kick sediment up so that particles can be read by the probe. Make sure that the person is far enough upstream so that an eddy is not created in the wake of the person.

    b. Using the top-setting wading rod, set the probe at 60% of stream depth, i.e., 60% of the way from the surface to the bottom. The wading rod is in meters and uses a Vernier scale. Each single bar on the rod indicates 2cm; the double bar is 10cm, and the triple bar is 50cm. So, to set the wading rod, for example, if the depth is at the mark above the triple bar the depth is 52cm, you then depress the lever to move the mobile rod to line the number 5 up with the number 2 on the Vernier scale at the top. The wading rod is now set at 60% of 52cm, or 31.2cm from the water surface.To begin measuring, set the location and depth on the SonTek datalogger at the starting edge. Once the edge is defined, you will need to move away from the edge and any interference created by the bank.

        1. Set the first level of measurement at a location away from the edge of the bank. Set the depth on the datalogger and adjust the wading rod.
        2. Move to the next interval, there should be about 20 increments along the width of the stream. Set the location and depth.
            - After two intervals, the SonTek should begin to "know" your interval, however, always check to make sure that it is right. You will always have to set the depth. It works better to move from low to high along the meter tape. Approve and warnings or repeat the measurement – depending on the warning and your judgment.

    c. Move along the tape to the next increment and repeat the depth and current measurements. Continue until the entire river breadth has been measured. (If the stream bottom is very inconsistent, you may have to take measurements at greater or less than the designated increment

along the tape; be sure to record these discrepancies in the notebook). Remove the tape after the transect is complete. Read the water depth again off of the staff gauge at the stilling well. The average of the depths before and after the discharge measurement is considered the river level for the given discharge.

d. The discharge is the sum of the products of each individual measurement (an individual measurement is the water velocity at a given increment times the depth at that increment times the distance from the previous increment). This can be computed easily in a spreadsheet.

e. If the SonTek was used, upon returning to the lab plug the SonTek into the laptop with the 9pin RS232 cable. Open SonTek FlowTracker software and connect to the SonTek on Comm Port 1. Save the file into the appropriate folder.

6. Water level will be recorded continuously in three ways: 1) with a pressure-sensing probe hooked up to a Campbell Scientific CR-10 datalogger, 2) with a Stevens float-and-pulley water level chart recorder, and 3) with an Onset HOBO water level logger.

   a. The CR-10 datalogger should have 8 fresh D batteries installed at the beginning of every summer; these should last for the entire summer. A CR-10 was deployed in Oksrukuyik Creek just downstream of the Dalton Highway until 2008. Beginning in 2009 the CR-10 was moved to upstream of the Dalton Highway due to road construction. Doug Kane's group (UA-Fairbanks) will deploy a CR-10 in the stilling well at the Kuparuk.

      1. Program the CR-10 datalogger for each parameter using the owner's manuals. FIRST MAKE SURE THE TIME IS SET PROPERLY ON THE DATALOGGER. The LTER computer has a directory called PC208w. Use the program EDLOG for program writing and editing. Transfer the program to the CR-10 keyboard and which can then be downloaded to the datalogger. The CR-10 Prompt Sheet is vital for using the CR10 keyboard and should be regularly consulted. Decide with Bruce Peterson the number of times per day the datalogger should record river depth. Consult the manual for each probe on how to wire the probes to the datalogger.
      2. Deploy the probes as soon as possible after arriving at Toolik.
      3. Doug Kane's group (UAF) will deploy the Kuparuk probes.
      4. At Oks Creek, place the datalogger inside the yellow weatherproof metal case with the hole in the bottom; the probes and their cables should be run through the hole. Using plenty of cable ties, mount the metal case on the wooden frame supported by rebar (about 5 m downstream from the stilling well). Drive a piece of rebar into the

stream bottom in at least waist-high water. Loosely attach a cable tie to the probe cables, loop the tie around the rebar, and slide the probes down so that they rest on the bottom. Place some large rocks on the cables so that the probes stay on the bottom and are somewhat protected.

5. Data will be downloaded into a storage module for transfer to the LTER computer at the Toolik Camp (both as an ASCII file and on a spreadsheet). You must bring the CR-10 keyboard to do this. Data should be downloaded and backed up weekly, especially in times of potential flooding. The datalogger should be removed if flooding is imminent.

6. Back at camp, use the 9-pin SC532 interface cable attached to the RS232 interface module to communicate between the storage module and computer. Use the SMCOM program (in PC208 directory) from DOS prompt to dump data. Select COM2 as the interface port. Select U for uncollected data and C for comma delineated file. This creates a *.DAT file which can then be imported into a spreadsheet. Keep the *.DAT files as backups. Place the *.DAT files in the directory set up for the specific site. Use the following template for naming downloaded files: YYCJULFL.dat where YY=year, C = site code, JUL is Julian day on which downloaded, and FL = file list; for example, 94N22401.dat is the datalogger file downloaded on Julian Day 224 for the new reach (Blueberry Cr.) in 1994.

b. A Stevens recorder is deployed in a stilling well upstream of the pipeline in the Kuparuk. The USGS had a Stevens recorder in a stilling well downstream of the Dalton Highway crossing at Oksrukuyik Creek until spring 2008 when it was removed due to potential road construction.

c. Onset HOBO water level loggers will be deployed in Oksrukuyik and Kuparuk. A barometric pressure logger should be left logging at the bank of the Kuparuk River near the water level Onset HOBO or in the Wet Lab at Toolik Field Station. These loggers will be overwintered to determine freeze and thaw dates. The first year of use was the winter of 2008-09. The HOBOs fit inside pipes with end caps that have holes drilled to allow contact with the water. The pipes are then fitted to conduit that can fit over rebar that is pounded into the river bottom. Leashes should also be fitted to secure the HOBO to the bank to insure they do not get broken away during peak snow melt and ice flows. A staff gage is also attached to the conduit to determine stage height.

1. The HOBO should be “calibrated” at the beginning of each season. Launch the HOBO using HOBOWare Pro – be sure the time is set

exactly to GMT. Partially fill a bucket of water with lake water. Place the HOBO in the water. Measure and record the depth of water. Let the HOBO equilibrate for 15 minutes. Add more water, noting the time. Measure the depth of water again and let equilibrate. Offload the data and determine that the time and depth of water is the same in the HOBO as what you manually recorded.

2. Using the HOBO requires the HOBO shuttle and a computer with the HOBOWare software. To launch the HOBO connect the HOBO to the shuttle and the shuttle to the computer. Open HOBOWare and click launch device. A time delay can be set for when logging should begin so that all loggers can begin at the same time – this makes data crunching easier. The time should be set exactly to GMT. For summer deployment the HOBO should log every 10 minutes. For winter deployment logging should occur once an hour so that the battery lasts until it can be retrieved in the summer.

3. To upload data from the logger in the field use the shuttle – unscrew the black cap and line up the flat end of the logger with the arrow on the shuttle and press the lever. Once the light on the shuttle is green the data has been uploaded. The logger can then be put back in the stream to continue logging.

4. To download the data from the shuttle, connect it to the computer using the USB cable. In HOBOWare click Readout Device. All of the data will be collected from the shuttle into files for each HOBO. Each file can then be renamed and saved into an appropriate folder. The data will be cleared off the shuttle. File saving and management is important here!

7. Stream height should also be recorded as often as possible by reading the river depth on the staff gauge and recording it, along with the time (AST) and date, in the notebook stored in the stilling well (and on the chart recorder in the stilling well box; write the time, date, and river level on the recorder paper and draw an arrow to the spot at which the chart recorder needle is currently located). These data are used to calibrate the manual discharge measurements with the river height data from the datalogger.

8. At the end of the season, construct a discharge curve for the stream.
   a. Convert the stage heights from the manual discharge measurements (i.e., the measurements taken in part A of this section) from feet (the measurements taken from the staff gauge) to meters (the depth measurements recorded by the datalogger at the same time and date as the manual discharge measurements).

b. In Excel (5.0 or higher), plot the 6 (or more) discharge measurements from part A of this section: stage height in meters on the x-axis, discharge in cubic meters per second on the y-axis.

c. Use the trendline function to draw the line that best fits the discharge data. You should select for a power curve and select for the equation and r^2 value to be displayed. (Hint: Be sure to display the numbers in the equation to several decimal places so that your computations in the next steps are precise.)

d. The equation produced by the relationship will probably be in the form of: $y = a(x^b)$, where y is discharge in m^3/s, a and b are coefficients, and x is stage height in m.

e. Now you can calculate the discharge over the course of the season, simply by plugging in the depth readings from the datalogger measurements as the x variable in the equation. Create a discharge column in the spreadsheet containing the datalogger depth measurements, input the formula using the equation (substituting the depth measurements for x). Then, plot the discharge value against the date. This will give you a summer discharge profile.

F. TSS

1. Precombust the filters at 450℃ for 4 hours in a muffle furnace to remove any organic carbon on the filter surface.
2. Cool filters by storing in a desiccator overnight to prevent moisture build-up on the filter surface (Or place in an 60℃ dryin g oven for about an hour).
3. Measure the masses of the filters are measured on a 4 or 5-place balance and record.
4. Store the filters in petri dishes with a label on which an identification number (e.g. TSS09-001), and spaces for the recording of the station, date, volume filtered is printed (See example below).
5. Record in the field book the identification number of the filter and the volume of water that was filtered. Also record this information on the petri dish label. Keep the filter in this dish until you get back to lab.
6. If you do not want to filter in the field, take a 2L sample of the water back to the lab and us a vacuum set up to filter the water. *Remember to record the filter number and volume filtered.*
7. Place the filter(s) into a numbered, pre-weighed aluminum weigh boat(s). Record both the filter number and the weigh boat identification in a lab book.
8. Place the weigh boat(s) into the 105℃ drying ov en for at least twelve hours. Cover the boat(s) with a sheet of aluminum foil to prevent foreign material from falling onto the filter surface.
9. Place the boat(s) into a desiccator to cool. Record the weight of the room temperature filter + weigh boat.

10. Place the boat(s) into a ***preheated and stabilized***, 550℃ muffle furnace for two hours.
11. Cool filters + weigh boat(s). Depending on the muffle furnace demand at camp, either a) remove hot filter + weigh boats, and allow them to cool in a GLASS desiccator, or b) turn off furnace and cool filter + weigh boat(s) in furnace. Once filters are cool enough to handle, record the room temperature weight of the filter + weigh boat.
12. Discard the filter.
13. Calculations
    a. Using the six measurements below the total suspended sediments and loss on ignition can be calculated.
        - Tare weight of the dry, pre-combusted, numbered, unused filter, (W_{filt}, mg)
        - The volume of water filtered (V_{filt}, ml)
        - Tare weight of the dry and empty weighing tin W_{tin}, mg)
        - Tare weight of the 105^{o}C dried tin plus used filter (W_{dry}, mg)
        - Tare weight of the 550^{o}C combusted tin plus used filter (W_{comb}, mg)
        - Tare weight of the combusted empty tin(W_{post}, mg)
    b. Calculations of TSS and LOI
        - TSS = (W_{dry} – W_{tin} - W_{filt}) / (V_{filt}/1000) in mg TSS/L
        - LOI = (W_{dry} – W_{comb}) / (V_{filt}/1000) in mg LOI/L
        - Weighing check = (W_{post} – W_{tin}) / (W_{tin})] * 100 or % change in tin weight

G. Dripper

1. Phosphoric acid barrels should be ordered in April of each year because they must be special ordered and take a while to get to Toolik. Once they are shipped to Fairbanks ask the Logistics coordinator to hold them in Fairbanks until June to prevent freezing. Unused barrels should be shipped to Warm Storage for the winter.
2. Set up on the Kuparuk is done on 25 June every year. From 1985-1995 the dripper was at .56k, from 1996-2010 it was at 1.4k, from 2011 to present there are two drippers: Big Drip at 0.0k, and Little Drip at 1.4k. A helo flight should be scheduled before 25 June to sling the phosphoric acid barrels to 0.0k in a fish tote, as well as the pump, batteries, and all other materials needed. The dripper at 1.4k is walked from the parking area at the bridge.
3. The dripper pump should be checked upon arrival to camp. The pump should also be stored in Warm Storage for the winter.
    a. check that all of the tubings fit and are long enough – use Teflon tape to seal the screws to prevent air from getting in the line
    b. check the timing – the dripper at 0.0k, “Big Drip” should drip at 2.4mL/min, and the dripper at 1.4k, “Little Drip” should be dripping at 1.2ml/min. – you can just use water to get it started

c. Using the tubes and rope set up the dripper line so that the phosphoric acid drips into the middle of the river. Use rebar and knots to get the line taught and high over the river – the higher the better so that it is less likely to get taken down in a high water event
d. check the dripper every day to make sure that there is plenty of acid and it is dripping at the right rate. The acid barrel should be replaced ~1x/week

H. Macroinvertebrates

1. Surber samples
   a. Using the standard Surber sampler collect two samples from each macroinvertebrate collection station. Use a scrub brush to stir up the sediment into the net of the Surber sampler. Rinse the sample out of the net into a bucket. Pour the sample through a micron sieve. Pour the sample into a whirl pack – large ones are necessary in the fertilized reach because there will be a lot of moss in the samples.
2. Drift samples
   a. record the number on the current meter, attach it to the drift net
   b. set up the drift net perpendicular to the direction of flow
   c. record the time
   d. measure the height of the water on the net
   e. after 15 minutes, take the net down, record the current meter number
   f. rinse the sample into a bucket, and then through a sieve (same as with the Surber sample), pour sample into a whirl pack
3. preservation
   a. preserve all samples in the lab with ~10mL of formalin in the hood in Lab 3.

I. Moss point transects

1. field equipment
   a. 50m tape
   b. view scope
   c. field notebook and pencil
2. methods
   a. Attach the free end of the field tape to one handle of the viewscope. The other person (the ‘recorder’) plays the tape out at 20cm intervals and simultaneously records the ‘hit’ identified by the caller.
   b. Record the date, the start time (HH:MM), the station name, the interval (20cm for all stations in the Kuparuk), the direction of movement (TR>TL or TL>TR), and the relative location of the transect above the immediate previous transect (e.g. +5m or +4 m).
   c. Develop a simple alphanumeric key so that you can efficiently record species without having to write down a lengthy name. It is most efficient if two people work in the field as a team, with one person moving across the stream calling out the observed cover types and one person on the shore to record the observations.

d. Record the 'hit' codes in the field book in columns. Start recording down the page then jump up and to the right, going down a new column until the transect is completed.
e. At each station, do a minimum of 5 (five) transects across the stream, spaced evenly along the riffle. A spacing of 4-5 m works well for most stations.
f. Note that if most stations are on the order of 20 m wide, a 20 cm interval along transects will produce 100 points per transect and 500 points for a station with 5 transects. This is a desirable sampling density.
    - The point observations must be converted to percent cover (C%), as follows:

$$C\% = (Ni / Nt) * 100$$

where Ni is the number of observed points that match the class type i (hits) and Nt is the total number of points observed. We use an Excel spreadsheet to organize and automatically analyze the data.

3. moss biomass

J. Fish

1. Permits

Permits must be applied for in the spring through AK Fish and Game. A report must be filled out with specifications for what fish work will be done. A general Toolik Area permit is always needed, other supplemental permits may also be required (i.e. Anaktuvuk River Fire). If other parts of the project (i.e. Lakes) will be doing any fish work be sure to have them fill out the information for their section. IACUC forms are also needed each year. Some years it is just a renewal, other years an entire permit must be written.

The AK Fish and Game permit requires that a state biologist must be contacted (email is fine) before any fish work begins. Post-season reports must also be filed, including a data report of all fish caught and a completion report with data and analyses for all fish work. The data report is due 31 January of each year, the completion report is due 1 June of each year.

2. Young of the Year (YOY)
    a. Field equipment
        - dip nets
        - labeled YOY containers
        - YOY hats
        - polarized sunglasses

- cooler with ice
- seine
- battery aerators
- backpack electrofisher
- salt
- orange insulated gloves

b. In the field
   - At each station try to catch 10 YOY. YOY typically emerge earlier on Oks than on the Kup by about a week. When they are small they are usually found in slow moving backwaters and side channels and are easier to catch. As they get bigger they move to faster waters. The seine can be used to try to corral them into a smaller area. It is easier to find them on sunny days, they often just don't come out from under rocks if it is cloudy. There are many techniques that can be used – get creative and be patient. Do not spend more than half an hour at any station.
   - The electrofisher can be used when they are bigger, but you need to add salt to the water to raise the conductivity enough for it to work. **Make sure everyone is wearing orange gloves and waders that don't leak before operating the electrofisher.**
   - When you catch them, place them into the containers with water, then set the container in the stream (with a rock on top) to keep the water cool. When you get back to the truck place the containers in the cooler with ice. The battery powered aerators can be used to keep the water oxygenated.

c. Return to the lab with live fish (check with Linda if that is the protocol for the year, some years the YOY are kept for genetic information). Most of the weighing and measuring equipment is in Lab 3.
   - To weigh and measure the fish they should be anesthetized with clove oil. At the beginning of the season make a dilution of clove oil to be used for the summer.
     - Put some water in a weigh boat, add one or two drops of clove oil. Put one or two YOY into the weight boat until they are anesthetized. Take one out at a time and measure with calipers. Then place on the balance to weight. Record the length and weight of each YOY.
     - To help the YOY recover put them in a weigh boat with fresh water and a drop or two of Stress Coat.
     - Freshen the water in the clove oil and Stress Coat dishes as needed
   - Return all YOY to the appropriate river, including any casualties, per the Alaska Fish and Game research permit.

3. Adult grayling
   a. Field equipment

- spin reels and rods (check condition at the beginning of the season, some may need replacing) or fly rods
- mepps spinner lures sizes #0 and #1 with barbs depressed and two of three hooks cut off, or flies
- mesh holding bags
- mesh holding pens – 4x4x4 nylon-mesh with 4 rods of rebar per pen to anchor pen in the stream
- Field balance (check batteries)
- pliers/Leatherman tool
- Weigh boat
- fish measuring board
- Square bucket
- Five gallon buckets (several)
- Sun shade
- Kit with tagging equipment
  - syringes with rod plunger and tagging needles
  - tags – check the protocol for the season – ½ duplex, full duplex, Floy juvenile tags
  - BioMark tag readers – reads both half and full duplex
    - check batteries and that it is working properly before going in the field
  - MS-222 for anesthetizing
  - baking soda to raise the pH of MS-222 solution
  - betadine solution – to sanitize the tagging needles between fish

b. What to do in the field – Fish!!
   - there are several reliable areas – ask Linda or Bruce, they know them well
   - Set up the tagging equipment – keeping at least two 5 gallon buckets – one for MS-222 and one for recovery, use the sun shade to help protect the area from wind and as a staging area for supplies
   - Each person fishing should also have a 5 gallon bucket to hold and carry any catches to the person tagging
   - When each fish is caught anesthetize it
     - tag it – for further tagging information see the PIT Tag Marking Procedures Manual from the Columbia River Basin Fish and Wildlife Authority
     - measure total length of fish to the nearest 0.1cm from tip of nose to bottom lobe of caudal fin (if caudal fin is missing or damaged mention it in field notebook)
     - measure wet weight to nearest gram using the portable field balance
     - record tag number, length, weight, and location in the field book, as well as any other appropriate comments

- Place the fish in the stream. Hold the fish by the caudal-peduncle, head into the current, until the fish has had an opportunity to recover from the anesthesia. Release the fish.

- if there are several fish at once keep some in the mesh holding bags while others are being worked up
- if fish are to be weighed on an empty stomach, keep them in the holding pen overnight – return the next day to tag and weigh
- if stomach contents are being analyzed
  - While it is still anesthetized, hold the fish with its mouth over the catchment pan.
  - Fill a 60-cc syringe (loaded with a 13-gauge needle, tipped with rubber tubing to prevent scratching) with water and carefully insert tubing down the fish's esophagus.
  - Inject the water, forcing the fish to egest its stomach contents through the mouth and into the pan. Gentle pressure on the fish's stomach helps to induce egestion.
  - Filter stomach contents through the 100-µm nylon mesh.
  - Transfer the stomach contents from the mesh into a 250-ml plastic bottle (use the funnel to make this easier). Label and preserve stomach contents in 95% ethanol.
  - Place the fish in the stream. Hold the fish by the caudal-peduncle, head into the current, until the fish has had an opportunity to recover from the anesthesia. Release the fish.
  - Return stomach contents to camp. Store in a sturdy box and ship or hand-carry to RESL for analysis.

c. weir
   - set up

II. Lab Methods at Toolik

The "nutrient" RA helps in Wet Lab to make reagents and standards for everyone. These procedures include the methods used by the Nutrient RA as well as the procedures followed to analyze samples.

A. Soluble Reactive Phosphorus

The method used for soluble reactive phosphorus determination is based on Parsons et al. 1984. It involves the reaction of phosphorus with molybdate, ascorbic acid, and trivalent antimony. The molybdic acids are reduced to a blue-colored complex which is then read for absorbance on a UV spectrophotometer. A 1 cm cell is used in the spectrophotometer.

1. **Mixed Reagent for Phosphate Analysis:**

This MUST be made the day you want to use it – it is only stable for 6 hrs. Solns *a*, *b*, and *d* can be prepared ahead of time in bulk. Soln *c* must be made daily. Use pre-labeled bottles, volumetrics and graduated cylinders.

ADD TO CLEAN CALIBRATED (500 uL) RE-PIPETTER in the following order:

a. 50 mL Ammonium para Molybdate Solution

b. 125 mL Sulfuric Acid Solution

c. 50 mL Ascorbic Acid Solution *MUST BE FRESH*

d. 25 mL Potassium Antimony-Tartrate Solution

This will be enough for 500 samples.

2. **Stock Solutions:**

There are a set of bottles/graduated cylinders that are reused to make these solutions. Keep them labeled and don't use for other purposes.

a. Ammonium para Molybdate Solution:
   - Dissolve 15 g Ammonium para Molybdate ($(NH_4)_6Mo_7O_{24}.4.H_2O$) in 500 mL DI water. Store in pre-labeled amber bottle – stable indefinitely.

b. Sulfuric Acid Solution:
   - **Safety glasses, gloves, lab coat and closed toed shoes required. Do not combine ingredients into a volumetric flask as the reaction is exothermic and will stretch the volumetric**

   - Add 70 mL of concentrated H_2SO_4 (Sulfuric Acid – 4.8 N) to 450 mL DI. Allow solution to cool. Bring to 1 L. Store in glass amber bottle.

c. Ascorbic Acid Solution – MAKE DAILY
   - Dissolve 2.7 g Ascorbic Acid in 50 mL DI (can use graduated cylinder)

d. Potassium Antimonyl – Tartrate Solution:
   - Dissolve 0.34 g Potassium Antimony in 250 mL DI water (warm if necessary). This solution is stable for many months.

3. **Standards**

a. Stock A Solution-1000mM PO_4-P

- Dissolve 0.136 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4, m.w. = 136.07) in approximately 900ml of deionized water contained in a 1 L volumetric flask (note all salts should be dried in oven prior to weighing).
- Dilute the solution to the mark with deionized water and mix it well.
- Transfer this solution to an amber bottle.
- Add 1ml chloroform for preservation. This solution is stable for many months. Refrigerate when it is not in use.

b. Stock B Solution-100mM PO_4-P

- Prepare this solution weekly.
- Using a volumetric pipet or a calibrated automatic pipet, add 10.0ml of Stock A to approximately 90 mL of deionized water contained in a 100ml volumetric flask.
- Dilute the solution to the mark with deionized water and mix it well.
- Add 1ml chloroform for preservation and store in an amber bottle.

c. Working Standards

- Prepare these solutions daily.
- Use adjustable, micro liter pipettes to add the designated volumes listed in the following table.
- Calibrate the pipet for each required volume. (NOTE: The standards for each day are presented in bold typeface. The other concentrations are included for reference, if needed.)
- Prepare each standard by adding the required amount of stock to the required volume volumetric flask containing deionized water.
- Standards for both Ammonium and Phosphate can be made in the same volumetrics.
- After adding standards for both methods, dilute each to the mark with deionized water and mix well. Keep these solutions tightly sealed.

4. Sample Prep Procedure

a. Sign up for a date and time on the door to the PO4 room specifying the number of samples and volume of reagent needed.
b. Make sure the sample tubes are clean and dry
c. Label the rack with tape and station names for ease when reading samples.
d. When ready to run samples, calibrate the pipette for 5mL
e. Rinse the pipette tip 3X's with DI
f. Rinse the tip 1X with sample
g. Pipette 5mL of sample into clean tube
    - run triplicates of each sample
    - Also run triplicates of check standards for every 10 samples
        - the Arctic LTER Streams group uses the Nutrient RA standard curve, but a standard curve can also be run if needed
    - Rinse 3X's with DI and 1X with next sample before each sample
h. Dispense reagent from repippeter a few times to make sure there is no air
i. Add 0.5mL of working reagent from the repipettor to each sample
j. Vortex each sample
k. cover with saran wrap
l. let sit for ~30-90 min in the light

5. Spectrophotometer reading (follow the Nutrient RA's instructions)

a. Make sure the waste tube is placed in correct waste container
b. Flush with degassed DI a few times before running samples
c. Read DI to get a stable number
d. Once DI is stable begin reading samples
e. Vortex samples before reading
f. Read each sample, recording the numbers in the notebook
g. If there are errors, check with the Nutrient RA about what should be done

6. Vial Cleaning

a. Pour samples into phosphorus waste container
b. Rinse tubes once with DI into the waste
c. Rinse tubes once with DI into the sink
d. (Remove tape from rack) put rack in acid bath
e. Rinse with DI 3X's
f. Dry upside down

B. Ammonium using the OPA method

1. Overview

The OPA method began being used in 1999. A year of comparison between the phenol method and OPA method occurred that year. After 1999, only the OPA method has been used.

Fluorescence is produced by the reaction of OPA with ammonium. Fluorometry is sensitive and simple so it seems to be a good way to measure ammonium, particularly at low levels. Details of methods, reagents, etc are given in Holmes *et al*. 1999 (CJFAS 56:1801-1808). This document supplements the manuscript and is intended to give a quick, user-friendly, informal overview of the procedure. It also details a variation of the method not discussed in the manuscript, which uses 2 mL sample and 8 mL working reagent.

a. Background Fluorescence

All samples auto-fluoresce to some degree. This BF must be subtracted from the observed sample fluorescence in order to quantify ammonium concentration. If it is found that BF doesn't change through the water column or down a stream transect it may be possible to take fewer BF measurements. If it does change however you will need to take a BF each time a sample is taken. In surface waters around Toolik Lake, ammonium concentrations tend to be very low and background fluorescence is relatively significant. Therefore, it is important to accurately quantify BF. In our limited experience so far, BF is relatively constant in a given water-body on a given day (for example, Toolik Main station or Kuparuk River transect), but BF varies across stations (and maybe temporally). Therefore, BF does not need to be sampled at every station within a given "station", but must be sampled at each stream. Another example: On June 23, 1999, BF was essentially constant at 11 Kuparuk River stations, but differed significantly in Hershey Creek, a small tributary to the Kuparuk River. If BF had not been measured in Hershey Creek and instead the Kuparuk BF was used, the Hershey Creek ammonium result would have been erroneous.

b. Matrix Effects

A summary of matrix effects are included below, but in 04 the lakes groups decided that the ME of Toolik Area lakes were close enough to DI that we could simply run a nutrient standard curve in DI and no longer utilize standard additions. The difficulty and inherent error of spiking with such small volumes of standard by unseasoned SRA's also led to this decision.

OPA and ammonium react differently in different waters. In DI water, a given amount of ammonium tends to produce more fluorescence than it would in lake or river or soil solution samples. To quantify ME and correct for it, standard additions are done to samples and compared to DI water standards. For surface waters around Toolik Lake, we have been spiking samples with 50 ul of 50 uM ammonium stock solution to quantify ME. In general, ME have been around 5-25 %. This correction is generally on the order of 0.01-0.03 uM for surface waters around Toolik Lake, but will be greater when ammonium concentrations are greater. Therefore it is important to note that for higher ammonium values a larger spike is required to assess ME. As with BF, ME appears to be relatively constant within a given water-body but will probably vary across stations and maybe temporally.

2. Reagents and Equipment

The reagents are made by the nutrient RA. Sign up for a time to react your samples and a time to read your samples. The OPA room should remain dark at all times. OPA is light sensitive.

The following is enough for about 48 liters of WR and 4 liters of Borate Buffer.

Reagent	Size	Sigma cat #
Sodium sulfite	250 g	S 4672
Sodium borate	2.5 kg	S 9640
Orthopthadialdehyde	100 g	P 1378
ETOH	2.5 l	

3. Preparation of Stock reagents

a. BORATE BUFFER (BB): Borate buffer without the sodium sulfite or the OPA is used to evaluate the sample background fluorescence (BF).

b. SODIUM SULFITE: Next prepare the sodium sulfite solution (2 g sodium sulfite to 250 mL DI water)

c. ORTHOPTHADIALDEHYDE (OPA): add 8 g OPA to 200 mL ethanol (keep this solution as dark as possible); shake vigorously until OPA dissolves.

d. WORKING REAGENT (WR): Working reagent appears to be stable for months, and its blank fluorescence decreases over time, so it is best to make WR in large batches and let it age. We make WR batches of about 4 L in 1 gallon brown Nalgene® bottles (these bottles actually hold about 4.4 L).

- To a clean 4-liter bottle (pre-react, or just rinse with DI if previously used for WR), add approximately 3 L DI. Then add 160 g sodium borate, cap, and shake vigorously until your arms are tired, then

rest, then do it some more, and add 20 ml of sodium sulfite solution to the 1 gallon jug with DI and sodium borate already added. Shake the jug some more. Finally, add 200 ml of OPA solution to the 4-liter jug. Shake some more, and then add DI until the bottle is nearly full – about 1 inch from the top. Shake a bit more, let age for at least a few days if possible, and then the WR is ready to use.

4. Fluorometer and filters

Optical Kit for Holmes NH4 Method
See web http://www.turnerdesigns.com/t2/doc/appnotes/s_0025.html
The Ammonium Optical Kit (P/N 10-303) includes two Near UV Mercury Vapor Lamps, 350nm excitation filter (310-390nm), a 410-600nm emission filter, a 1:75 Attenuator plate, and a 10-300 Reference Filter (>300nm). The 10-303 Optical Kit works in the 10-AU Digital Field Fluorometer, TD-700 Laboratory Fluorometer and the Model 10 Analog Fluorometer and also includes a reference filter (P/N 10-300).

5. Calculations

Four fluorescence values and the slope of a standard curve are needed to calculate uM NH4

- **a.** 1:4 DI H20/Working Reagent, DI WR
- **b.** 1:4 DI H20/Borate Buffer, (for to correct for background fluorescence) DI BF
- **c.** 1:4 Sample /Working Reagent, Samp WR
- **d.** 1:4 Sample/Borate Buffer, (for to correct for background fluorescence) Samp BF
- **e.** Slope of 0-3uM NH4 Std Curve

Calculating uM NH4

uM NH4= {Samp WR-(DI WR+(Samp BF-DI BF))}/{Slope of curve}

6. Procedure

a. Pre-reaction of vials (only need to do at beginning of season or with new vials):
   - New vials: Rinse 3x with DI, fill with 8 ml dilute working reagent and let sit for 3-24 hours.
   - Old vials: Rinse 1x with DI, fill with 8 ml dilute working reagent and let sit for at least 3 hours.

b. Dump WR into NH4 waste, rinse with 2-3 ml dilute (if available) or new working reagent

c. Fill with 8ml new working reagent.

d. Before setting up a run:
   - Dump old samples from vial into NH4 waste.
   - Rinse vials with DI, dump into NH4 waste

- Rinse vials with 2mL borate buffer, dump into NH4 waste
- Fill WR vials with 8 ml good working reagent. Use the same batch of working reagent for all samples and standards in your run.
- Fill buffer vials with 8 ml new borate buffer (don't need to rinse).

e. After you return from the field with your samples:
- Let standards and samples warm to room temp if not already.
- Pipette 2 ml of each standard (made by nutrient RA) into their respective vials (each standard and sample has one buffer vial and 2 working reagent vials).
- Change pipette tip after pipetting standards and before pipetting samples. You can use the same pipette tip for all samples, but rinse the tip 1x with DI and 1x with the next sample first.
- Pipette 2ml of samples into each row of numbered vials. Record which sample is in which row of vials.
- **Do all pipetting in the dark. OPA working reagent is sensitive to light.**
- Shake samples and store in the dark for 5-24 hours. Record the time the samples were shot up.

f. Reading samples-
- Verify that fluorometer is on – if not push the red button. Let it warm up for about 30 minutes before reading samples
- Use two tubes – one for buffer and one for working reagent
- Rinse 13x100mm borosilicate tube with DI
- Rinse 13x100mm borosilicate tube with a small amount of sample from scint vial – vortex
- Pour sample from vial into borosilicate tube - vortex
  - Rinse exterior of glass tube with DI and wipe with kimwipe/tissue to remove any fingerprints or residual WR.
- Place tube into fluorometer, replace black cap
- Allow reading to stabilize (about 5-10 seconds), press [*] and read average fluorescence value.
- reuse borosilicate tubes, rinsing 1x with DI and 1x with sample in between.
- Use new tubes if going from a high standard of sample to low.
- Use separate tubes for buffer samples and WR samples.

C. Chlorophyll a

1. Reading Samples on Turner Designs10-AU Fluorometer

TD-10AU should already be calibrated and ready for use, for more information regarding calibration, lamp/filter arrangements, etc. refer to users manual or Fluorometer use protocols.

*Safety Precautions, chronic exposure to acetone can cause health problems (see MSDS). Always wear gloves, eye protection, and make sure snorkel hood is working properly.

a. Verify TD fluorometer is on, if not press red button at front of unit and allow to warm up for 20 minutes.
b. Use the Chlorophyll notebook to record data
c. Place chlorophyll solid standard in fluorometer, replace black cap, wait 2 minutes for reading to stabilize, press [*] to read average and record values for both the high and low std. To switch between the two simply turn 180 degrees till solid slides into place. If readings deviate from mean std. readings by more than 10%, the unit may need recalibration (see Lakes or Nutrient RA).
d. Read and record resting fluorescence with no tube in fluorometer. It should be around 0.328. This will help you catch problems associated with changes in lamp intensity. A second check is a clean glass tube which has a fluorescence around 3.32.
e. Read and record fluorescence of two acetone blanks (90% acetone w/MgCO3) in 13x100 glass tubes
f. Reading samples - Carefully transfer extract from Falcon tube to a clean 13x100mm borosilicate tube (leave last 0.5ml of acetone, filter, and any particles in the Vulcan tube).
g. Wipe exterior of glass tube with kimwipe/tissue to remove any fingerprints or residual acetone.
h. Place tube into fluorometer, replace black cap
i. Allow reading to stabilize (about 5-10 seconds), press [*] and read average fluorescence value, record preacidification value (Rb).
j. Using calibrated pipette, transfer 300ul 0.1N HCL (or 100ul 0.3N HCL) into the sample you just read, mix by inversion (place Saran wrap over end, hold with thumb) or by using disposable glass pipette to “mix”
k. Let stand for 2 minutes (its easiest to work out a system where you read a row unacidified, then acidify and let stand while reading 2nd row, acidify, etc, etc)
l. Repeat steps 7-10, record post acidification fluorescence (Ra)
m. After recording both readings, dump contents of glass tube into acetone waste container, rinse with small amount of DI and acetone, let dry.
n. When finished with samples rerun and record low and high chl. solid standard values (let readings stabilize about 2 minutes)
o. Make sure all glass tubes are rinsed and disposed of properly, place cap on acetone waste bottle, clean up kimwipes, pipettes, acid, etc. Leave fluorometer on for next user
p. Remove filters from Vulcan tubes dispose into filter bin, rinse tubes with DI, and acetone.
q. See equations below to determine chlorophyll *a* and pheophytin *a* concentration.

Chlorophyll *a*

$C_{E,C}$ = Chlorophyll *a* concentration in the extract (usually 10ml)

$C_{E,C} = F_s(r/r-1)(R_b-R_a)$

Where:
$C_{E,C}$ = corrected chlorophyll *a* concentration (ug/L) in the extract solution analyzed
F_s = response factor for the sensitivity setting S
r = the before to after acidification ration of a pure chl a solution (Rb/Ra)
R_b = fluorescence of sample extract before acidification
R_a = fluorescence of sample extract after acidification

$C_{S,C}$ = corrected chl *a* concentration (ug/L) in the whole water sample

$C_{S,C}$ ={($C_{E,C}$ x extract volume in L x Dilution Factor)/(sample volume in L)}

Pheophytin *a*

P_E = pheophytin a concentration (ug/L) in the sample extract

$P_E = F_s\ (r/r-1)\ (rR_a - R_b)$

P_S = pheophytin *a* concentration (ug/L) in the whole water sample

P_S = (P_E x extract volume in L x dilution factor)/(sample volume in L)

Note: if no dilution took place, then DF equals 1

III. Lab Methods at the Rubenstein Ecosystem Science Laboratory (RESL)

A. Nitrate

QuikChem Method 31-107-04-1E for use on the Lachat (previously, QuikChem Method 31-107-04-1C was used)

This is a condensed version of the QuikChem method with details specific to working at the RESL on Arctic samples. It is advised to refer to the Lachat method for further information about this analysis. Samples are passed through a copperized cadmium column to reduce nitrate to nitrite. The nitrite (both original and reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-napthyl)-ethylenediamine dihydrochoride to form a highly colored azo dye which is measured colorimetrically.

1. Keep up to date notes after every use of the Lachat. This will help you to keep track of problems, and ways you have solved them in the past.
   Reagents – prep the day before getting started on the Lachat
   a. 15N sodium hydroxide
   b. 150 g NaOH into 250 mL water – the solution will get HOT – caution! Make it in the fume hood!
   c. Ammonium chloride buffer – pH 8.5
      - dissolve 85.0 g ammonium chloride and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate in a large beaker in ~800 mL water.
      - calibrate the pH meter with standards
      - adjust the pH of the ammonium chloride buffer solution by slowly adding sodium hydroxide while the pH probe is in the solution.
      - once everything is dissolved, pour into a 1L volumetric flask – rinse the beaker with DI into the flask to make sure all particles are in solution. Fill to mark with DI.
   d. Sulfanilamide color reagent
      - to a 1L volumetric flask add ~600mL water.
      - Sulfanilamide is light sensitive – wrap flask in aluminum foil
      - add 100mL 85% phosphoric acid, 40.0g sulfanilamide and 1.0g N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Stir to dissolve for 30 min. Dilute to the mark and invert to mix. Store in a dark bottle – it is stable for ~1 month.
   e. Nitrate and nitrite standards
      - To make primary standards (71.4uM N or 1000mg N/L)–
         - The primary standards for both $NaNO_3$ and KNO_2 need to be placed into an oven at 100C for two hours to burn off any moisture weight. All standards are made from the primary standard.

- Nitrate: Dissolve 6.04g $NaNO_3$ into 1 Liter of deionized water.
- Nitrite: Dissolve 6.048g KNO_2 into 1 Liter of deionized water

STD Label	Volume of stock (71.4uM diluted into 100mL	Concentration
A	100ml of Stock in 250ml Volumetric Flask.	28.6uM
B	50 ml of Standard A in 100ml Volumetric Flask	14.3uM
C	50ml of Stock	7.15uM
D	2.5ml of Stock	1.788uM
E	0.5ml of Stock	0.36uM
F	0ml of Stock (100ml DI)	0uM

- Determine cadmium column efficiency (efficiency is determined by comparing the nitrate to nitrite ratio. It was determined that a beginning column efficiency should not be less than 90%. Don't use the numbers after the column efficiency is below 85%).

2. Getting ready to run samples
   a. get samples out to thaw – a water bath can be used, or spacing them out on the bench
   b. Run DI through the tubes for ~10 min before running
   c. Load the method to be used – check the timings, concentrations, and DQM
      - the DQM is the QAQC for the program – it is good to run check standards once ~10-15 samples, including a column efficiency check.
      - Run replicates 1/10-15 samples as well to check the machine
   d. Load the table to be run – fill in the sample names and order
   e. Begin running reagents through the tubes
   f. Put the column on – be careful not to get air in it! Columns contain cadmium, which is toxic! Cadmium columns began being purchased (50237A from the Hach website) instead of recopperizing and packing because they can get ~1,000 samples, which saves time and contact with cadmium. The columns are stored in buffer – this seems to help keep air out and make them last longer.
   g. Run a standard curve to check baseline, standards, and column efficiency. If everything looks ok, samples can be run. If the samples are not ready yet, turn the column off and set the pump to minimum speed to reduce waste of reagents.
   h. To continue prepping the samples, pour samples into clean borosilicate tubes. We pour samples because the centrifuge tubes often break. 60mL of sample is available, so that if samples need to be rerun there is plenty of sample, however, this means that it can take a

while for the entire sample to thaw. Be patient, it is best if they are run at room temperature.

i. Before running the tray, make sure to clear the standard curve. Once everything is ready, hit Run tray (be sure to turn the column back on and set the pump to normal speed). Stay with the machine for a little while to make sure everything is running smoothly. Check to make sure the arm is working properly, check to make sure the tray is lined up correctly so the needle does not get out of line and cause air to be sucked.

j. If air does get sucked turn the column off immediately. If air gets in the column you can try to blow it through by pressing the max speed on the pump. If air gets in, stop the run, and let buffer run through for a little while – this sometimes help to work it out. Check the efficiency again, and try the run again.

3. If samples have very low concentrations of nitrate there are other methods available that have a lower detection limit, the one used has been historical.
4. If samples have negative concentrations spike additions can be run to determine if there is a matrix effect. If recovery is high (<95%) then it is likely that there is simply no nitrate in the sample.
   a. Spike additions to determine if there is sample interference
   - The volume of the spike additions also varied depending on the sample volume in the tube. Two equations are necessary:
     - (Sample volume) / (Stock concentration/ Desired Concentration) = Spike Volume
     - (Stock Concentration * (Spike Volume /(Sample Volume + Spike Volume))=True concentration
   - The first equation gives an approximate spike volume to add to the sample. The second equation gives the true concentration of the spike based on the approximate volume. The desired concentrations for this procedure are 0.50 µM for the low spike, and 2.0 µM for the high spike. The true concentrations were 0.49 µM and 1.92 µM, so for the 8 ml : 8ml example above, the low spike was 80 µL and the high was 320 µL.
     - In 2008 and 2009 spike additions were done (both samples and the standard curve were spiked)
       - A high spike had 0.5mL of 50uM standard added to 5.0mL sample to produce a concentration of 4.54uM
       - A low spike had 0.125mL of 50uM standard added to 5.0mL sample to produce a concentration of 1.22uM

B. TDP

The determination of total dissolved phosphorus is done by liberating organic phosphorus as inorganic phosphate through oxidation by persulfate. The total phosphate can then be determined using the molybdate method.

Stock Solutions – prepare ahead of time

Solution	**Concentration**	**Stability**
Ammonium heptamolybdate tetrahydrate	25g/250 mL DI	Stable for months in dark bottle, check for precipitate and stir before use
Potassium antimonly tartrate	5g/200 mL DI	Stable for months in dark bottle
Sulfuric acid	9 N H_2SO_4 = 250 mL 36 N H_2SO_4 made into 1L	
Acidified DI for working standards	0.012N = 12mL 1N HCl made into 1L	Use to make working standards
Primary phosphorus stock – potassium phosphate anhydrous	10,000 □M = 1.36g KH_2PO_4 to 1L DI	Stable for months refrigerated
Secondary phosphorus stock	50 □M P = 5mL primary stock diluted to make 1L	Stable for weeks refrigerated
Primary Sodium pyrophosphate decahydrate digestion check standard	1.33 g/ 1L = 10,000 □M P/L	Stable for weeks refrigerated
Secondary sodium pyrophosphate digestion standard	50 □M P = 5 mL primary stock diluted to make 1 L	Stable for weeks refrigerated
Ascorbic Acid solution for Working Reagent 1	2g ascorbic acid dissolved into 10 mL DI	Stable for the day: (ensure it is freshly made daily)

Digestion Solution - prepare fresh daily

Solution	**Contents**	**Ratios**
Persulfate solution	Potassium persulfate $K_2S_20_8$	2.5 g/ 50 mL DI Difficult to dissolve – use a little heat if necessary – DO NOT overheat

Standard dilutions – make fresh daily in dedicated 100 mL volumetric flasks, digestion check standards can be made in a similar way

Concentration	**Dilution from Secondary (2^o) 50 □M Phosphate Standard with acidified DI**
0.00 □M P	0.00 mL 2^o + 100 mL acidified DI
0.05 □M P	0.1 mL 2^o + 99.9 mL acidified DI
0.10 □M P	0.2 mL 2^o + 99.8 mL acidified DI
0.25 □M P	0.5 mL 2^o + 99.5 mL acidified DI
0.50 □M P	1.0 mL 2^o + 99.0 mL acidified DI
1.0 □M P	2.0 mL 2^o + 98.0 mL acidified DI
1.5 □M P	3.0 mL 2^o + 97.0 mL acidified DI
2.5 □M P	5.0 mL 2^o + 95.0 mL acidified DI

Concentration calculation $\mathbf{M_1V_1 = M_2V_2}$

(50 □M P 2^o stock solution)*(V_1) = (x Molarity)*(100 mL)

$$V_1 = \frac{\text{(x Molarity)*(100 mL)}}{\text{50 □M P } 2^o \text{ stock solution}}$$

Procedure

1. Prepare for Analysis:
    a. Create spreadsheet with sample info and label autoclave and regular racks
    b. Organize 100ml Volumetric Flasks to contain each concentration of the curve as prescribed in the "Standard Dilution" table above. Create dilution with check standards in the same fashion (typically of 0.1□M and 0.25□M).
    c. Put each of the standards in a prescribed beaker or sealable jar for easy pipetting
    d. Ensure test tubes for standards are labeled as the first seven on the rack. Include two digestion check standards in two additional test tubes after the standards.
2. Pipette 7.5 mL of standards, digestion check standards, and sample into Kimax screw top tubes
    a. Include a 0.0 uM P and mid-range check standard at the end of each sample rack row
    b. Include at least one lab replicate in each sample rack row
    c. Follow general pipetting rules – rinse tip with DI three times, rinse once with sample, pipet sample into tube
3. Add 0.188 mL potassium persulfate solution to each tube, vortex
4. Cap tubes tightly
5. Check levels of liquid in tubes – etch level on some
6. Fill bottom of autoclave with water

7. Check other liquid levels in autoclave
8. Adjust settings on autoclave – 90 min at 105^{o}C
   a. Place racks in tray
   b. Autoclave for 90 min
9. Remove tubes from autoclave and let cool
   a. Refrigerate if not running until the next day

Working Solutions – prepare fresh daily – lasts about six hours
**** Be sure to use chemicals in correct concentrations as labeled on first page ****

Solution	Contents	Ratios
Working Reagent 1 Adjust volumes as needed COVER IN FOIL!!	9 N Sulfuric acid solution and ascorbic acid solution	1:1 10 mL 9 N sulfuric acid + 10 mL fresh ascorbic acid solution (2g/10ml)
Working Reagent 2 Adjust volumes as needed	9 N sulfuric acid solution: Ammonium molybdate stock: potassium antimony tartrate stock	70:25:4 14 mL 9 N sulfuric acid + 5 mL ammonium molybdate solution + 0.8 mL potassium antimonl tartrate solution

10. Run standard curve
    a. Add .15ml WR1 – vortex
    b. Add .15ml WR2 – vortex
    c. Stand 30 min (covered with foil or saran wrap, but in the light)
    d. Run on spec at 885nm
11. When standard curve is acceptable → run samples
    a. Add .15ml WR1 – vortex
    b. Add .15ml WR2 – vortex
    c. Stand 30 min
12. While samples are reacting
    a. Make non-digested calibration curve (don't need organic standards or to acidify)
       i) Pipette 7.5ml potassium phosphate standard solution into clean vials
       ii) Add .15ml WR1 – vortex
       iii) Add .15ml WR2 – vortex
       iv) React for 30 min
13. Run samples on Spec
14. Run non-digested curve on spec.
15. Clean all volumetric flasks, beakers, and sealable jars. If reusing volumetric flasks or sealable jars for another day, clean and fill with DI water. Let DI water sit until glassware is used again.

Chemicals list

- Potassium persulfate ($K_2S_2O_8$) – J. T. Baker 3239-01
- Potassium Phosphate (KH_2PO_4) – Fisher P285-500
- Hydrochloric Acid – Trace Metal Grade – Fisher
- Sulfuric Acid – Trace Metal Grade – Fisher
- L-Ascorbic Acid – Fisher A61-100
- Potassium Antimony Tartrate $C_8H_4K_2O_{12}Sb_2*3H_20$ – J.T. Baker 0864-4
- Ammonium Molybdate $(NH_4)_6Mo_7O_{24}*4H_20$ – Fisher A674-500

Supplies

- Volumetric flasks for standards
- Sealable jars for standards
- Pipettes
- Pre-made solutions to make working reagents
- Clean tubes, racks, caps

C. TDN/DOC

1. Beginning in 2005 the Shimadzu TOC-L was used for TDN and DOC. Previously; TDN was determined by persulfate digestion and analyzed on the Lachat.
2. The TOC-L uses carrier gas, which is used to supply oxygen at 150mL/min to the combustion tube filled with oxidation catalyst. This tube is heated to 680 degrees C and samples are burned in the combustion tube to form carbon dioxide. The carrier gas, containing the carbon dioxide created from the combustion, flows into a dehumidifier, where it is cooled and dehydrated. The gas then goes through a halogen scrubber before it reaches the cell of a non-dispersive infrared NDIR gas analyzer. This analyzer detects the carbon dioxide, and the analog detection signal of the NDIR forms a peak. The area of this peak is measured by a data processor, and the peak's area is proportional to the concentration of the TC in the sample. To detect TN, the sample also goes through a combustion tube at 720 degrees C. The sample decomposes at this temperature to become nitrogen monoxide. The carrier gas carries the nitrogen monoxide to a dehumidifier, where it is dehydrated. Then it enters a chemiluminescence gas analyzer, where the nitrogen monoxide is detected. The detection signal from the analyzer genterates a peak which are proportional to the concentration of the TN in the sample.
3. Running the Shimadzu TOC requires a combustion column, O2 tank, and standard.
   a. The combustion column may need to be repacked with reconditioned pellets. The supplies should be in the drawer next to the machine. A sample column is available to know how much of each layer is required. Quartz and glass cotton are used at each end of the column.

PROCEDURE

1. Prepare Needed Stock Standards.
   a. Primary Stock Solution: 160,000uM C, 20,000 uM N.
      i. To prepare, add 4.08g KHP and 2.02g KNO3 to DI water. Dilute to mark with DI water.
   b. Secondary Working Solution.
      i. Add 10ml of Primary Stock Solution to DI water. Dilute to mark with DI water.
2. Prepare TOC for Analysis.
   a. Open Air Gas, take note of gas pressure. Ensure it is at least above 600psi to prevent running out of gas mid-run. Check the pressure on the tank and the flowmeter in the machine to make sure flow is appropriate.
   b. Add Secondary Working Solution to the small loose tubing coming out of the left hand side of the TOC. This sampling tube is known

as “Vial 0” to the computer program. There should be another piece of tubing going to the ASI auto sampler.

c. Check to make sure adequate levels of acid and DI water are present within the analyzer. Fill as needed. Check the manual to see what should be checked daily, and occasionally. Ensure that everything is ready for analysis.
d. Turn on TOC and ASI auto sampler, allow it to warm up.

3. Open the “TOC-L Sample Table Editor”.
4. Create a New Sample Table.
5. Insert a “Conditioning Sample”. This will help “warm up” the TOC to get ready for analysis.
    a. Right click on the first cell on the Sample Table.
    b. Click Insert-Sample
    c. Choose “20120216_CN_ebs.met” as the Method.
    d. Continue by clicking “Next”, through the current settings. Current Settings should be set as: Manual Dilution:1; No. of Determinations:1; Units:mg/L; Injection Volume:150; Expected Conc. Range: 400; SD Max:0.1000; CV Max:2.00%; No. of Washes: 2; Auto Dilution:1; Sparge Gas Flow:90; Sparge Time: 01:30; Acid Addition: 1.5%. Click Finish when at end.
    e. Right Click on Sample Row Number. Go to Measurement Settings.
        i. Click on the NPOC tab, Change the Number of Dilutions from 3/5 to 15/15.
        ii. Click OK.
6. Insert the TOC calibration curve for the run.
    a. Right click on the second cell on the Sample Table.
    b. Click Insert- Calibration Curve.
    c. Choose “NPOC_19mgL.cal”. Click Open.
7. Insert the TDN calibration curve for the run.
    a. Right click on the third cell on the Sample Table.
    b. Click Insert- Calibration Curve.
    c. Choose “TN_2.8mgL.cal”. Click Open.
8. Insert Samples
    a. Right click on the forth cell on the Sample Table.
    b. Click Insert-Multiple Samples
    c. Choose Choose “20120216_CN_ebs.met” as the Method. Click Next.
    d. Choose Number of Samples to insert. Keep in mind that you will also need check standards and DI samples throughout the run. Click finish.

9. The Vial Settings tab will open up. Change all vial numbers for the conditioning sample, and standard curves to “0”. Do this by clicking on the cell and hit enter after each entry. After completion, click OK.
10. Enter sample numbers into the spreadsheet, in the place which they will be within the ASI-L auto sampler. It is suggested that after each 10 samples, a check standard and DI water test should be analyzed. Ensure that all samples are poured into the vial and then it is sealed with the lid and septum. DI should be within the ASI-L auto sampler with the samples. The check standard should be set as “Vial 0”, and should be set to an auto-dilution of 8 times. As you enter samples you will have to change the Vial numbers in which they will be. You will have to go to the vial settings to adjust these numbers. The vial settings can be accessed by clicking the “birthday cake” like icon, which is supposed to look like the ASI-L auto sampler, in the upper right hand side of the Sample Table Sheet.
11. Connect to the TOC-L analyzer within the program. Click on Monitor to ensure that the TOC has warmed up to the appropriate temperature and the proper amount of air pressure going into the system. If all parameters are met, click on “Start”. Choose if you want the analyzer to shut down or stay on after analysis, and click “Start”.
12. Allow the Analyzer to complete. This may take a few hours or overnight depending on how many samples are being analyzed.
13. Acid Wash all TOC vials, and then ash the vials at 500 degrees Celsius for 2 hours.

D. Particulate Phosphorus

1. Particulate matter collected on a glass fiber filter is ignited at low temperature to destroy organic matter. The ignited filter is heated with dilute HCl, which extracts the phosphorus and converts it to ortho-phosphate. The phosphorus is then analyzed by a version of the reactive phosphorus method.

   a. 1 N HCl – 82.64ml of 12.1 N HCl made to 1000 ml with DI (you will need some for flushing the spec also)

   b. Ammonium Molybdate
   - In 500ml volumetric add 15g of ammonium paramolybdate, $(NH_4)_6Mo_7O_{24}$ *$4H_2O$ and approximately 350ml DI water, shake/stir till dissolved, make to volume (500ml) with DI and transfer to plastic amber bottle (transparent bottle can be used, just keep out of sunlight).

   c. 4.8N Sulfuric Acid (H_2SO_4)
   - In ice bath Add 140ml of 36N H_2SO_4 to 900ml DI water. Allow solution to cool and store in a glass bottle.

   d. Ascorbic Acid Solution
   - In 500ml volumetric add 27g ascorbic acid and approx. 350ml DI water, shake/stir till dissolved, make to volume with DI (500ml). Store in plastic bottle in freezer. Thaw for use but refreeze. Stable for many months but should not be left at room temperature. Tip: separate solution into plastic disposable scint vials or similar containers, in this way you can thaw out only the amount needed.

   e. Potassium Antimonyl Tartrate (careful, toxic)
   - 0.34g potassium antimonyl tartrate, fill to volume with 250ml DI water, warm if necessary. Store in glass or plastic, stable for many months.

   f. Mixed Reagent 500ml (have samples ready, make daily, only keeps 6-8 hours)
   - Final amount can be modified depending on number of samples. Reagent should be made from the following proportions in the order given.

Volume	Reagent	Comment
100ml	Ammonium Molybdate	
250ml	4.8N Sulfuric Acid	
100ml	Ascorbic Acid Solution	Should turn pale yellow color
50ml	Potassium Antimonyl Tartrate	

1. PROCEDURE

a. Dry filters in petri dishes (with the lids slightly open at 70º C for 1 hour, I typically dry the blank and standard filters in bulk in tin foil.

b. Prepare standards and blanks by placing ashed GF/F filters in marked reaction tubes and adding appropriate amount of primary stock standard (see below). Stock is added directly to blank/std tubes which contain filters.

- The following is a table of amount of primary (1000uM) stock to add, and µmols of PO_4 in each tube after standard is added:

1º Std (µl)	µmols PO_4
0	0.0
25	0.025
50	0.050
100	0.10
150	0.15
200	0.20

- Sometimes the filters don't absorb all of the liquid very well – try to put it directly onto the filter and let it sit for a while in the drying oven. If it doesn't get fully absorbed make a note.

b. Cover each tube with foil

c. Place tubes in muffle furnace at 500º C for 1 hour

d. After sample has cooled, add 2 ml of 1 N HCl and 10 ml of DI, Vortex

e. Cap samples tightly and place in oven at 104º C for 2 hours

f. After samples have cooled, add 2.5 ml of mixed reagent (see above) to each tube, Vortex

g. Re-cap and place tubes in the dark

h. Allow 30 minutes for color reaction to occur, std should be noticeably blue, and read standards and samples at 885 nm on a spectrophotometer within 2 hours.

i. Obtain a regression equation y=mx+b where y is the absorbance, m is the slope of the linear regression, x is the µmols of PO4, and b is the intercept. Calculate the amount of PPO_4 in each sample tube (in µmols) using this standard curve:

µmols = (y - b) / m

The concentration of PPO_4 in the water sample (in µmols/L) is then determined by dividing the µmols by the amount of water filtered for each sample (in liters).

E. CHN

These samples are analyzed in the Jeffords Building at UVM with a CN auto analyzer.
The FlashEANC Soil Analyzer is calibrated with aspartic acid, and uses glycine as a secondary QC. It uses a combustion method to convert the sample elements to the simple gases (CO_2, H_2O, and N_2). The sample is first oxidized in a pure oxygen environment; the resulting gases are then controlled to exact conditions of pressure, temperature, and volume. Finally, the product gases are separated. Then, under steady-state conditions, the gases are measured as a function of thermal conductivity. A known standard is first analyzed to calibrate the analyzer in micrograms. The calibration factor is then used to determine unknowns. All quantitation is performed on a weight percent basis, using a gravimetric technique. The system uses a steady state, wavefront chromatographic approach to separate the measured gases. This approach involves separating a continuous homogenized mixture of gases through a chromatographic column. As the gases elute, each gas separates as a steady state step, with each subsequent gas added to the previous one. Consequently, each step becomes the reference for the subsequent signal.

It is good to have some filters pelletized before beginning so that you can run samples at the same time as pelletizing. Pelletize standard K factors and blanks first.

1. On the day before the filters are to be pelletized, place them in a drying oven at 37C for at least 12 hours. Then transfer the dried filters to a dessicator. Pull a vacuum on the dessicator if possible.
2. On the day of use sign into the log book
3. Check the pressure on the helium (20 psi) and oxygen (16 psi) tanks.
4. Check that the printer is on. Check the carousel autosampler and set it to position 1
   a. make sure the printer paper is loaded properly and that it will not roll over on itself (pull it forward so that it will feed to the floor)
5. Turn the gas saver off – press Parameter key and type 22. Press 2 for off.
6. Purge by pressing the PURGE GAS key. Purge helium for 100 seconrds and Oxygen for 30 seconds.
7. Check the settings to make sure it is set for filters
   a. Parameters 29
   b. Water concentration – yes
   c. Filter PPM
   d. Filter volume – unless all samples were the same volume input 1000mL – can process data to correct for actual amount filtered
8. Check to determine how many more samples can be run on the reduction column and the filter collector – don't plan on running more than it is set for
9. Take the filters to the instrument room. Clean the working space with ethanol. Get the tin disks and forceps from the drawers beneath the CHN

analyzer and the balance. Clean the forceps thoroughly with kimwipes and ethanol. **Use the forceps to handle the tin disks and the filters; do not use your fingers!**

10. To pelletize a filter, center the filter on a tin disk, sample side up. Make sure you have only one tin disk; they're very thin and it's easy to pick up more than one at once. With the forceps, fold the disk in half so that the filter is on the inside. Fold the disk again lengthwise (you should now have a long thin strip rather than a quarter-circle; see the manual).
11. Fold the ends of the strip over, and then roll the strip into a sausage or jelly roll shape. If you roll it up too tightly, you may rip the tin, so be careful. When it is rolled up, no part of the filter inside should be visible.
12. Place the "sausage" (jelly roll if you're vegetarian) into the pelletizing cylinder. Put the cylinder in the widemouth-side of the stage mount. Pull down the handle part way and line up the press with the opening of the cylinder. When they are lined up, pull the handle all the way down and press the sausage.
13. Lift the handle and remove the cylinder. Flip the stage mount over so that the narrow-mouth side is up. Put the cylinder back on the stage, line up the press and the opening, and pull the handle down. The press will knock the pellet into the narrow-mouth opening.
14. Using the forceps, remove the pellet and place it into a pellet-holding tray (there are several trays in the instrument room). **It is essential that you keep track of which pellet is in which hole in the tray.** The holes in the trays are marked (A1, A2, A3, etc.), so use the datasheets provided (titled "AUTO RUN Sample Information (*Filters*)") to record which pelletized sample is in which hole. Leave the first row (A1-A12) empty; you will put your initial standards and blanks in that row. About every 10 pellets, skip two holes (a standard blank and a filter blank will go in these holes).
15. Calibrate the microbalance in the corner, using the instructions provided in the booklet on top of the balance. The calibration weights are in the drawer under the balance. The range setting should be 20 mg.
16. Pack standard blanks (also called a K factors):
    - **a.** Place an ashed 25-mm GF/F filter on a tin disk, fold them in half with the forceps, and then unfold partially so that there is a crease down the middle of the filter and tin.
    - **b.** Place a counterweight pellet on the left pan of the balance (there are several counterweights in a small plastic box; however, some were made with more than one tin disk and will cause an error on the scale, so keep trying until you get the counterweight with only one tin disk).
    - **c.** Place the creased filter and disk on the right pan of the balance, lower the pan arrest, and zero the balance. Raise the pan arrest.
    - **d.** Using the spatula, place a small amount of acetanilide on the creased filter. Lower the pan arrest and check the weight. Add or remove acetanilide until you have between 2 and 3 mg of acetanilide. Record the weight on a Preliminary Standardization Data Sheet (available in folder on table in the CHN room).

e. **VERY CAREFULLY,** using the two pairs of forceps, roll up the disk into a sausage as described above. Make sure you don't spill any of the acetanilide and that you don't rip the tin. **Start over** if you do either of these things, or else the weight in the next step will not be correct.
f. Pelletize the sausage and reweigh. If the weight is greater or less than the original weight by more than about **0.010 mg**, start over. If not, then record the second weight.
g. Still using the forceps, place the pellet in a designated hole in the pellet tray. The first five K factor pellets will go on row A (in holes A1, A3, A5, A7, and A9). Subsequent K factor pellets will go within the samples; as you recall, every ten samples or so, you left two holes blank. The first of these holes is for a K factor. So, place the K factor pellet in the first of a pair of empty holes and record the hole number and the weight of the acetanilide in the K factor pellet on the data sheet (use the Preliminary Data Sheet if the pellets are going into row A and the Auto Run Sample Info data sheet if the pellets are being implanted within the samples).

17. Pack filter blanks:
    a. Using the forceps, place a blank 25-mm GF/F filter that you brought back from the field onto one of the tin disks.
    b. Pelletize the blank filter in the same manner as the sample filters (described above); do not add acetanilide.
    c. Transfer the pelletized blank filter to the pellet tray. Place in one of the empty holes designated for blanks. The first four blanks will go on row A (in holes A2, A4, A6, and A8). Subsequent blanks will go within the samples; as you recall, every ten samples or so, you left two holes blank. The second of these holes is for a blank. So, place the blank pellet in the second of a pair of empty holes and record the hole number on the data sheet (use the Preliminary Data Sheet if the pellets are going into row A and the Auto Run Sample Info data sheet if the pellets are being implanted within the samples).
18. At this point, you are ready to run your samples. If, however, the CHN machine is already in use or not ready to run, tape your pellet trays shut, label them, and place them in a dessicator with activated dessicant and draw a vacuum on them until the machine is available.
19. When the machine is available, check with Marshall Otter to make sure that there is enough helium, oxygen, and nitrogen gas, and that the columns are fresh enough to run all of your samples.
20. When you are ready to start a run begin with Kfactors and blanks to calibrate the machine.
21. Run an unweighed conditioning sample of acetanilide as a single run sample
    a. use 2-3mg acetanilide
    b. press SINGLE RUN key
    c. when prompted by 1 BLANK 2 KFACT 3 SAMP type 3 to select sample
    d. when promted, type in sample ID and estimated weight

e. press START to begin

22. Next, run 3 oxygen blanks
    a. press SINGLE RUN key
    b. select 1 for blanks
    c. when prompted for the number of blanks type 3
    d. press START to begin
    e. Blank values should fall within these ranges
        - C blank = 10-50 +/- 30
        - H blank = 100-300 +/- 100
        - N Blank = 50-120 +/- 16
23. After blank runs, alternate between K-factors and filter blanks
    a. run using the SINGLE RUN key
        - for K-factors select 2
            - Acetanilide is type S1
            - type in the second weight of the K-factor
            - press START to begin
    b. If K-factor varies more than a 0.5% from the default or previous running mean an error will appear. If they are not ok additional K-factors and blanks should be run
24. Once the running mean for K-factors is acceptable, you can begin running samples
    a. A K-factor and filter blank should be run after every 10 samples
        - If the K-factor is out of tolerance after samples, run more K-factors and filter blanks
25. For your samples you can use the AUTO RUN
    a. Place your samples in the holes in the carousel – lining up the numbers with your datasheet so that you know what each sample is
    b. Press AUTO RUN
    c. begin typing in the names of the sample
    d. when you get to a Kfactor remember to type it in as a S1 kfactor and enter the weight
26. You can begin running and continue to add to the AUTO RUN as you complete packing more samples
27. Keep an eye on the machine – make sure that it isn't going out of tolerance because that can indicate that the reduction column is done and you can lose samples, also make sure that the carousel is moving properly and the pellets aren't getting caught when it turns
28. Errors – check the manual to help determine issues and how to fix it
29. Shut down
    a. fill out log book
    b. turn gas saver ON
    c. set time and date
    d. Make sure CHN is left in Standby
    e. clean up

F. Anions

These samples are analyzed in the laboratory in Jeffords Hall on campus with the ion chromatograph. Samples pass through a pressurized chromatographic column where anions are absorbed by column constituents. As the eluent, a liquid that extracts ions, runs through the column, ions begin separating from the column. Concentrations of certain ions (such as Cl^{-1} and SO_4) are determined by their retention time as they are separated from the column.

1. Materials
   - **a.** Dionex chromatographer
   - **b.** Stock solutions for Cl^{-1} and SO_4^{2}
   - **c.** 7 100-ml volumetric flasks
   - **d.** Nanopure water
   - **e.** 100- and 1000-l pipettes (calibrated), tips
   - **f.** 7 125 ml poly bottles to store standards

2. If Total Anion concentration is < 600 um the Dionex can be set up to run on the Fast column (~ 3 min / sample). Make sure that the Dionex has plenty of regenerant and eluent.
   - **a.** Regenerant (2 liters)
     - 100 ml .5 N H_2SO_4 (27.7 ml H_2SO_4 in 2 liter DI)
     - Fill to 2 Liters with DI
   - **b.** Eluent (2 liters) Should be relatively fresh.
     - .4240g **Na_2CO_3** and
     - 0.0252 g **$NaHCO_3$**
     - Fill to 2 liters

3. Standard stock solutions for chloride and sulfate can be made in the same volumetric
   - **a.** Chloride = Sodium Chloride NaCl (FW = 58.44)
     - 10 mM NaCl = .05844 g / 100 ml
   - **b.** Sulfate = Sodium Sulfate Na_2SO_4 (FW = 142.04)
     - 10 mM Na_2SO_4 = .142. g / 100ml

4. Standards:
   - **a.** 2 *u*M Cloride & sulfate = 20 *u*l 10 mM NaCl & 20 *u*l 10 mM Na_2SO_4 /100 ml DI
   - **b.** 5 *u*M Cloride & sulfate = 50 *u*l 10 mM NaCl & 50 *u*l 10 mM Na_2SO_4 /100 ml DI
   - **c.** 10 *u*M Cloride & sulfate = 100 *u*l 10 mM NaCl & 100 *u*l 10 mM Na_2SO_4 /100 ml DI
   - **d.** 15 *u*M Cloride & sulfate = 150 *u*l 10 mM NaCl & 150 *u*l 10 mM Na_2SO_4 /100 ml DI

e. 30 *u*M Cloride & sulfate = 300 *u*l 10 mM NaCl & 300 *u*l 10 mM Na_2SO_4 /100 ml DI

5. The attenuation will need to be changed for the different concentrations in the standard curve. On the fast column the following work well. For actual area under the curve multiply the attenuation by the area.

Standard	*concentration (uM)*	*attenuation*
1	2	1
2	5	1
3	10	3
4	15	3
5	30	10

6. To run a sample:

a. Inject sample into port (>1 ml)
b. Hit [Load/ Inject] (valve will switch)
c. Change attenuation if needed
d. Plot sample [Shift/Down] [Plot] [Enter]

G. Cations

Rains, Theodore, C. 1984. Atomic Absorption Spectrometry. Water Analysis, Vol II Edited by Roger A. Minear and Lawrence H. Keith.

These samples are analyzed in the laboratory in Jeffords on campus with an Absorption/ Emission Spectrophotometer.

1. Materials:
   - **a.** Reference standard solutions for atomic absorption ± 1%:
   - **b.** Na^{+}, (1000 ppm 100 ml poly bottle Fisher cat # SS 139-100)
   - **c.** K^{+}, (1000 ppm 100 ml poly bottle Fisher cat # SP 351-100)
   - **d.** Mg^{+2}, (1000 ppm 100 ml poly bottle Fisher cat # SM 51-100)
   - **e.** Ca^{+2} (1000 ppm 100 ml poly bottle Fisher cat # SC 191-100)
   - **f.** 20 100-ml volumetric flasks (5 per element)
   - **g.** 5-ml volumetric pipette & pipette bulb
   - **h.** 1000-*ul* pipette (calibrated), several tips for dilution's
   - **i.** deionized water
   - **j.** HCl (**trace metal grade**)
   - **k.** 10% Lanthanum Cloride ($LaCL_3$ FW 371.38) (for calcium)
   - **l.** Absorption/Emission spectrophotometer
   - **m.** clean, dry scintillation vials (at least one per sample)
   - **n.** 10 ml Re-pipette

   - **o.** Samples should be preserved by acidifying to ~ pH 2 (100 *u*l Ultrex HCL / 60 ml sample.

   - **p.** Sign up for use of the Absorption/Emission Spectrophotometer in the Instrument Room
   - **q.** The week that you plan to run your samples, make standards using stock solutions kept in the back room of the Aquatics Lab, second floor, Ecosystems. For each ion, make standards in concentrations of 1, 2, 3, 4, and 5 parts per million (ppm). Make the standards in 100-ml volumetric flasks (labeled) under the hood. *Use trace metal grade HCl, measured in the volumetric pipette.

     - 1.00 ppm = 100 *u*l stock, 5 ml HCl, water to fill line.
     - 2.00 ppm = 200 *u*l stock, 5 ml HCl, water to fill line.
     - 3.00 ppm = 300 *u*l stock, 5 ml HCl, water to fill line.
     - 4.00 ppm = 400 *u*l stock, 5 ml HCl, water to fill line.
     - 5.00 ppm = 500 *u*l stock, 5 ml HCl, water to fill line.

IMPORTANT NOTE:

1. Chemical interference in flame AAS originates during dissociation of the analyte in the flame cell forming a compound that volatilizes at a different rate than the standard. One method of alleviating this type of interference is

through the addition of a releasing agent or protective chelate. A releasing agent is a metal or salt that forms a more stable compound with the interferent than the analyte. For calcium analysis the addition of lanthanum, which acts as a releasing agent and reduces interference with the calcium peak from Al, Si, PO_4^{3-}, and SO_4^{2-} is required.

1.1. Lanthanum Cloride ($LaCL_3$ FW 371.38) should be added to a 1% final concentration to all samples and standards. 10% $LaCL_3$ = 37.13 g / liter.

1.2. For each calcium standard, add 10 ml of 10% lanthanum solution
   1.2.1. 1.00 ppm = 100 *u*l stock, 5 ml HCl, 10 ml of 10% $LaCL_3$ water to fill line.
   1.2.2. 2.00 ppm = 200 *u*l stock, 5 ml HCl, 10 ml of 10% $LaCL_3$ water to fill line.
   1.2.3. 3.00 ppm = 300 *u*l stock, 5 ml HCl, 10 ml of 10% $LaCL_3$ water to fill line.
   1.2.4. 4.00 ppm = 400 *u*l stock, 5 ml HCl, 10 ml of 10% $LaCL_3$ water to fill line.
   1.2.5. 5.00 ppm = 500 *u*l stock, 5 ml HCl, 10 ml of 10% $LaCL_3$ water to fill line.

1.3. For each 1 ml of sample add one of the following depending on concentration.
   1.3.1. 1 ml of (200 ml 10% $LaCL_3$ in 1 liter DI) multiply reading by 2 for final conc.
   1.3.2. 9 ml of (Add 100 ml 10% $LaCL_3$ to 900 ml DI) multiply reading by 10 for final conc.

2. Fill the large beaker (inverted on the front of the spec) with DI water (in the carboy on the shelf to the left of the gas cylinders). Turn on the exhaust fan (switch by the door).
3. When it is time to run the samples on the Absorption/Emission spec, fill out the log book in the Instrument Room (on the little table next to the AAS. Start up procedure varies depending on the elements being run. Emission methods are used for K^+, Na^+ analysis, while absorption methods are used for Mg^{+2}, Ca^{+2}. For absorption you will need to fill out the log book for lamp and Continuous Operating current (Mg^{+2}, Ca^{+2}. 15 ma).
4. Turn on the air (the knob is on the wall above the gas cylinders). Then, turn on the acetylene. The low stage should stay above 15 (in red), and the high stage should not fall below 70 psi. If a new tank is needed, call Martha @ 7272).
5. Initial set up for all elements.
   5.1. Push [Power on].
   5.2. Make the following control settings:
      5.2.1. SIGNAL - LAMP
      5.2.2. GAIN - Fully counterclockwise
      5.2.3. BG Corrector - AA

5.2.4. LAMP current control - fully counterclockwise

5.3. Set the SIGNAL control to set up. Set the SLIT control to the setting appropriate for the element of interest. Adjust with the course adjust wavelength control to obtain the wavelength of interest.

5.3.1. K+, wavelength = 766.5 and slit width = 0.7.

5.3.2. Na+, wavelength = 589.0 and slit width = 0.2.

5.3.3. Mg^{+2} wavelength 285.2 and slit width .7

5.3.4. Ca^{+2} wavelength is 422.2 and slit width is .7)

5.4. Push [Flame on]. If an error message appears (E 50) it is due to air in the lines, push [Flame on] again.

5.5. Put the sipper tube into the DI in the big beaker.

5.6. Adjust the ratio of air/fuel so that it is about 18:40.

6. Emission methods (use for K^+, Na^+ analysis)

6.1. If a lamp is installed be sure to unplug it at this point.

6.2. Turn the [Signal] knob to EM (emission)

6.3. While aspirating a blank solution press [AZ] to auto-zero the spec.

6.4. Aspirate the most concentrated standard solution, optimize the wavelength by adjusting the [Fine adjust] dial slowly back and forth to provide maximum energy. Adjust the gain control as necessary to keep the LAMP /ENERGY display on scale.

6.5. Pick two standards between which samples fall (range of 1 ppm). While sipping the higher of the two standards adjust GAIN to provide a reading of about 75 on the LAMP/ENERGY display. Note that every time standards are changed the gain must also be changed.

6.5.1. NOTE: Calibration curves for flame emission measurements may not be accurate over extended concentration ranges

6.6. Change the integration time to 3 seconds by hitting 3 [t],

6.7. Set values for the standards: hit 1.00 and [s1] and 2.00 [s2] for 1ppm and 2 ppm if two decimal places are desired use two.

6.8. Hit [az] to auto zero while sipping DI. Sip low standard and wait for absorbance to stabilize and hit [s1] repeat for standard 2

6.9. Sip s1 again to check reading if not similar redo calibration

6.10. Run all standards in a given range while periodically checking high standard to be sure gain has not dropped if it has either edge it back or reset gain to 75.

7. Absorption (Mg^{+2} , Ca^{+2})

7.1. Place correct hollow cathode lamp in lamp holder and plug in.

7.2. Turn the lamp current control until the LAMP/ENERGY display shows the proper lamp current, as given on the lamp label for continuous operation (continuous operating current for Ca^{+2} & Mg^{+2} lamp is 15).

7.3. Turn signal knob to set up. Turn the FINE ADJUST wavelength control slowly to obtain a maximum reading on the LAMP/ENERGY display. Use the

gain control to adjust the maximum reading to 75. If the gain should become too high, an over range reading of EE will be obtained.

7.4. Align lamp by turning the two alignment knobs on the lamp holder to maximize the LAMP /ENERGY display reading. Again use the gain control to make the maximum reading 75.

7.4.1. NOTE: As the lamp warms up, the display value may increase slightly drop gain back down to final setting of 75

7.5. Close the lamp compartment door.

7.6. Wait ten minutes

7.7. Set the SIGNAL control to concentration.

7.8. Hit [az] to auto zero while sipping DI. Sip low standard and wait for absorbance to stabilize and hit [s1] repeat for standard 2

7.9. Sip s1 again to check reading if not similar redo calibration

7.10. Run all standards in a given range while periodically checking gain and readjust to 75 as lamp warms

8. Shut down
   8.1. after last sample let run for 10 minutes
   8.2. turn signal, gain, lamp counter clockwise
   8.3. fill out log book
   8.4. turn flame off
   8.5. pull sipper out of DI and dump DI
   8.6. shut off air and acetylene
   8.7. empty lines of air and acetylene by hitting [check 0-2] [check fuel]
   8.8. turn off machine
   8.9. turn off fan
   8.10. unplug lamp

H. Alkalinity

GRAN TITRATION

Principle:

Alkalinity is the measurement of the Acid Neutralizing Capacity (ANC) of a water sample. Alkalinity is usually reported in units of milliequivalents per liter of sample (meq/L). In Toolik area waters, ANC is due primarily to HCO_3, CO_3^{-2}, OH^-, and certain organic bases. Of these, HCO_3 is usually by far the most important species. We are interested in measuring alkalinity for a couple of reasons. When coupled with a measurement of pH, alkalinity can be used to compute total dissolved inorganic carbon (needed for primary production measurements), and the partial pressure CO_2 gas in the water (useful for atmosphere-water interaction studies). In waters which have a near-neutral pH (most of the Toolik area), alkalinity correlates well with the total concentration of dissolved ions, and hence can be useful in categorizing the overall ionic state of a water sample. Also, when used as a long-term monitoring tool, it can detect acidic impacts on lakes and rivers. Alkalinity is usually measured by titrating a water sample with a strong acid. The alkalinity is a measurement of the amount (equivalents) of acid needed to exactly neutralize the original ANC of the water sample. There are two common methods of performing this titration; both are based on monitoring pH as acid is added to a water sample. The procedure outlined below is based on the "Gran" methodology. In the Gran method, a series of pH measurements are made. The alkalinity is determined by an analysis of the rate at which pH changes in response to acid additions. In practice, the alkalinity is computed with a computer program written for this purpose.

Collection and storage of water samples:

Either plastic or glass sample bottles are acceptable. If sample bottles were previously acid-cleaned, then bottle should be rinsed thoroughly with sample water if possible to insure that sample is not exposed to acid. Samples CANNOT be treated with either acidic or basic preservatives. This includes all acids and bases, and many preservatives such as formaldehyde. Samples should not be frozen. Analyses should be conducted within 24 hours. EITHER filtered or unfiltered water may be used for analyses.

EXCEPTIONS:

1. If there is going to be a delay of more than one day between collection and analysis, then filtering is recommended. Alkalinity can change if biological activity results in either the production or dissolution of organic particles. If samples are going to be kept for more extended periods, then they should be stored in completely full, tightly stopper bottles.
2. If water samples are suspected of containing particles of calcium carbonate, sample MUST be filtered before analysis. If the sample is

turbid, but composition of particles is not known, then filtering is an advisable precaution.

3. Anoxic water samples present special problems. Anoxic water samples from the Toolik area usually contain high concentrations of dissolved iron. When the anoxic water comes in contact with oxygen from the atmosphere, the dissolved iron will begin to precipitate. Iron precipitation strongly decreases alkalinity, and must be avoided. The best solution is to collect anoxic water samples by completely filling to overflowing GLASS dissolved oxygen bottles. Bottles must remain tightly stoppered until analysis. Bottles should be opened individually JUST BEFORE analysis is to begin.

pH calibration for the Mettler Auto-Titrator:

1. Turn on the Mettler Auto-Titrator, switch is on the back located on the lower left corner .
2. Remove the gray cap covering the opening of the pH tube, near the top of the pH tube.
3. Press "reset".
4. Press "1" then "Elec Calib".
5. On the screen you will see a small red light beside buffer A.
6. Fill a Mettler cup with approximately 50-ml of 4.0 buffer solution.
7. Screw the Mettler titration cup in place.
8. Press "start", the stirrer should turn on if not check that the stirrer knob is set to 3.
9. Once the Mettler has calibrated the 4.0 buffer, the red light will light up beside the buffer B on the screen.
10. Empty the 4.0 buffer back into its container, rinse with DI and wipe dry the Mettler cup, and fill with the approximately 50ml of 7.0 buffer solution. Screw the Mettler cup in place.
11. Press "start", the stirrer should turn on.
12. The Mettler Auto-Titrator will beep once it is done calibrating and usually "0" will appear on the screen once it is ready.
13. Pour the 7 buffer solution back into its container, rinse and dry the Mettler cup.

Prior to running samples:

1. All samples should be at room temperature when analyzed because pH is strongly influenced by changes in temperature.
2. The laboratory room where the measurements are made must be free of acid and base fumes. These vapors will dissolve in water and change the alkalinity. Likewise, if samples are filtered or otherwise processed in the laboratory, care must be taken to avoid contamination.
3. The methodology described below presumes that the alkalinity analyses will be made using the Mettler “Auto-Titrator.” The pH electrodes should be calibrated at least once per day as described in

pH calibration section above, usually when the Mettler titrator is turned on. The normality of the acid used for titration MUST be known very accurately. The acid routinely used at Toolik is 0.1 N H_2SO_4, and is usually purchased commercially. However, other normalities may be used if necessary.

4. Before beginning analyses, make sure acid dispensing and delivery tubes on the titrator are free of air bubbles. This can be a common problem at the start of each day and after the burette is refilled. Bubbles can usually be removed by gentle tapping to free the bubble from the tubing walls, followed by the dispensing of several mL of acid to flush the bubble from the tube. The dispensing of acid is done my entering a value, e.g., 2 or 5 and pressing the "dose ml" button.

Running the alkalinity titration

1. Between samples, rinse the electrode 10 x with the DI pump and then wipe dry the pH electrode, stirrer and Acid tube with a kim wipe to remove all water droplets.
2. Special Mettler titration cups are used for the samples. Titration cups MUST be clean and completely dry before use. Fill the titration cups with a KNOWN volume of sample. 50-mL samples are routinely used for Toolik measurements; however, other volumes may be used. The accuracy of the alkalinity measurement depends DIRECTLY on the accuracy of the sample volume. The preferred method for measuring and dispensing sample volumes is a volumetric pipette. The second choice for measuring and dispensing samples is a graduated cylinder. Make sure to rinse the pipette with DI or sample rinse between samples.
3. Screw the titration cup into the electrode/stirrer/dispenser holder.
4. Turn on the stirrer, knob on left side of Mettler, and set to 3.
5. Begin alkalinity titration by pressing the following keys on the Mettler:
   5.1. Press “pH meas” , the stirrer will turn on and watch the pH rise to 7-8. Occasionally the samples pH will not reach 7 in which case continue to the next step, but wait at least 2 minutes.
   5.2. Press “Reset”
   5.3. Press “ENDPOINT” to set pH endpoint for initial titration. Enter a value of 4.1 and press "ENDPOINT” again. If the Mettler is already set to 4.1, you need only press "ENDPOINT" once.
   5.4. Press “START” four times to begin titration. MAKE SURE stirrer is on and is at a moderate speed (about 3).
   5.5. Titrator will beep when the endpoint has been reached.
   5.6. *Write down amount of acid added*
   5.7. To obtain the pH of the sample once the endpoint has been reached, press "pH meas", stirrer should come on. Allow sample to stir for at least one minute. This allows CO2 to degas from the sample and permits stable pH measurement. Once the pH stabilizes write down the pH, if you have difficulty in determining the pH with the stirrer on, you can turn off the stirrer by turning the knob and write down the pH value before the

values start to increase. The pH values will start to increase as soon as the stirrer is turned off.

5.8. At this step in the analysis, pH should be between 3.95 and 4.05. If pH is >4.05, reset ENDPOINT for 4.0 and proceed again as above. When proper pH range has been achieved, record BOTH mL and pH.

5.9. Press “RESET,” enter 0.02, and press “DOSE ml.” This will cause 0.02 mL of acid to be dispensed into the titration cup.

5.10. Press "pH meas", the stirrer will turn on. After the stirrer has been on for 1 minute or once the pH stabilizes write down the pH, or you can turn off the stirrer by turning the knob and write down the pH value before the values start to increase. Record BOTH pH AND the TOTAL volume of acid, which has been added to that point.

5.11. Press “DOSE mL” to dispense more acid and repeat as above. A pH change of about 0.1 pH unit is desirable. It will probably be necessary to increase the amount of acid dispensed as the titration proceeds such as using 0.02 ml, 0.03 ml, 0.04 ml, 0.05 ml, 0.06 ml, 0.07ml, if you are close to a pH of 3.1 and still need to add acid to get 10 points you can add smaller amounts like 0.01ml..

5.12. Continue until at least eight readings have been determined which cover the pH range of approximately 3.0 - 4.0. Again, it is necessary to record BOTH the pH and TOTAL volume of acid dispensed for each set of measurements.

6. Compute alkalinity by entering data into Quattro Pro spreadsheet Alkalinity program. Data entered for each sample are 1) sample volume; 2) acidic normality; 3) sets of volume and pH measurements. Alkalinity is computed by running the macro program which is part of the spreadsheet. Check the output file for Correlation Coefficient and Predicted Acid Normality. If Correlation Coefficient is not greater than 0.98, or if Predicted Acid Normality differs greatly from actual acid normality, then there has probably been an error in the titration or data entry.

Alkalinity Calculation on Spreadsheet:

Example of data entry in spread sheet:

Sample	Hershey Cr. St.4, 500m	
Date collected	23-Jul-01	
Date run	26-Jul-01	
Acid N	0.1005	
Sample V (ml)	0.05	
Acid (ml)	PH	[H]
0.3471	3.994	0.000101
0.3671	3.867	0.000136
0.3871	3.771	0.000169

0.4071	3.69	0.000204
0.4371	3.585	0.00026
0.4771	3.484	0.000328
0.5171	3.395	0.000403
0.5671	3.31	0.00049
0.6271	3.224	0.000597
0.6971	3.14	0.000724

END-POINT (L)	0.000291
R^2	0.999939
ALK (meq/L)	0.585914

[H]: 10 ^ (-1*(acid at 4.1 endpoint)
E.g., 10^(-1*0.3471) = 0.000101
END-POINT: intercept (all acid points, [H] points) / 1000
E.g., intercept (0.3471…0.6271,0.000101…0.000724)/1000 = 0.000291
ALKALINITY: [(endpoint*acid N) / sample volume] * 1000
E.g., [(0.000291*0.10005) / 0.05]* 1000 =0.585914

Comments:

1. Do not let the pH probe sit in the air, if you will not be running samples for a prolonged period, screw in place a Mettler cup filled with 60 ml of DI water.
2. When finished using the Mittler Auto-titrator remember to place the gray cap back onto the pH probe
3. Turn off the Mettler Auto-titrator at the end of the day.
4. Occasionally the screw that holds the pump into place may become loose and thus the Mettler Auto-titrator will be titrating an incorrect amount of acid. Check that the amount of acid titrated is the amount entered, if it isn't check the screw under the pump platform.
5. RUNNING STANDARD
   5.1. run a known alkalinity standard to test the acid used. Run these standards whenever a new acid is used.

IV. Appendix A – Formerly Used Methods

A. 2x2 rock scrubs

1. The following methods are based on methods from the LTER database, previous protocol sheets, and Peterson et al. (1993), and have been modified into outline form.

2. Materials
3. At each station, rinse the wash basin, scrub brush, slide holders, and wash bottle 3X with river water. Fill the wash bottle with river water.
4. From a riffle, select 5 (or 3 if only doing 3 reps) rocks that fit the following criteria:
   a. rocks with no filamentous algae or moss (to eliminate overestimates of chl due to filamentous algae or moss)
   b. rocks with fairly smooth upper surface (uneven surfaces prevent efficient removal of epilithon)
   c. rocks that have been submerged for a long period of time.
   d. It is possible that very few rocks at some stations will meet all of the above criteria. If you must select rocks that do not fit any or all of the criteria, make careful and thorough notes describing the deviations.
5. Place the slide holder over a smooth portion of the upper surface of the rock. With the brush, scrub the area within slide holder. Hold the rock over the basin so that all scrubbate falls into the basin.
6. With the wash bottle, rinse the scrubbed area, the holder, and the brush into the basin. Pour any remaining rinse water into the basin. **Record the initial slurry volume used** (volume of rinse bottle).
7. Pour the contents of the basin into a labeled (by river and station) centrifuge tube or bottle. Use the funnel to facilitate pouring.
8. Repeat steps 2-8 for each rock at each station. Only scrub one rock per sample bottle at each station. You will end up with 3-5 replicate slurries from each station (depending on how many reps you decide to do).

B. Epilithic primary productivity

1. In addition to the studies involving the rock scrubs, rocks will be incubated in special laboratory chambers to determine primary productivity of the epilithic algal layer. The amount of photosynthesis and respiration occurring on individual rocks will be determined during the incubation period. These rocks may have filamentous algae; the rock scrubs excluded such rocks when possible.

2. The methods below are based on methods written by Breck Bowden, the PI for this project, in the document files of the Arctic LTER database in 1989 and 1990 (filenames 89BOMETA.DOC and 90BOMETA.DOC). They have been modified into outline form.

   a. Materials:
   - carboy (optional)
   - coolers

3. At the scheduled time (determined at the beginning of the field season), collect rocks at random locations within pools or riffles at

each sample station (locations vary from year to year). Select surface rocks with a "typical" development of epilithon, based on visual inspection of the station, for study. Reject rocks with heavy moss growth and instead choose rocks of a uniform size and shape, that would fit neatly in the chamber bottom.

a. In the Kuparuk River, 3 to 4 rocks of a modal shape and size essentially covers the chamber bottom in a single layer, at a surface density similar to that found in the river. Substrate size in Oksrukuyik Creek is substantially smaller than in the Kuparuk River; thus, use 4 to 7 rocks from Oksrukuyik Creek to cover each chamber bottom. (Substrate sizes for New Reach streams will be determined at the beginning of the summer.)

b. Collect water for the incubations in carboys (optional; Toolik Lake water may be used instead).

c. Place rocks collected from the river in coolers, without water, to keep them cool and moist. (If the rocks are kept submerged in water in the coolers, delicate epilithic material will be dislodged during transport from the field to the lab. Without water, the rocks and epilithon can be transported with the epilithon essentially intact, even with flocculent pool epilithon.) The time from collection and transport to installation in a chamber is generally 1-2 h, and should be minimized.

d. Upon return to Toolik, immediately place rocks and water into the experimental chambers in Bowden's polar tent.

C. Bioassays of epilithic algae

1. Bioassays of epilithic algae are done using small-scale artificial nutrient-diffusible substrata. These bioassays produce many replicates of numerous treatments at minimal cost and time expenditure.

2. The methods below are based on Gibeau and Miller (1989) and have been modified into outline form. Mike Miller is the PI for this project.

3. Materials:
   a. agar vials (see below)
   b. porous porcelain discs, soaked in HCl (see below)
   c. wooden vial holders (see below)

d. silicon sealant
e. rope to secure vial holders (should be several meters longer than width of stream)
f. 2 metal spikes per wooden vial holder
g. pliers (bring when time to remove vials from river)
h. 125-ml urine cups (one per vial) (bring when time to remove vials from river)

4. Three assay experiments will be conducted in each stream during each summer. The dates and sites will be determined at the beginning of the field season. Each experiment lasts three weeks.
5. Each experimental chamber is composed of a 10-dram plastic vial (Dynalab Corp. #2636-0010) used as a reservoir, filled with various nutrient-supplemented agar treatments.
6. The agar treatments are 37-ml of a 2% (w/v) Difco Ultrapure Agar solution augmented with one each of the following treatments:
   a. control (plain agar)
   b. humic acid extract plus phosphorus (2 g humics/L and 0.5 ml conc. HCl plus 0.005 moles K_2PO_4/L)
   c. phosphorus (0.005 moles K_2PO_4/L)
   d. ammonium (0.05 moles NH_4Cl/L)
   e. phosphorus plus ammonium (0.005 moles K_2PO_4/L + 0.05 moles NH_4Cl/L)
   f. vitamins (B_1 0.1 mg/L, plus Biotin 5 mg/L)
   g. a trace metal mixture (Woods Hole formula plus 0.0999g NTA/500 ml as a chelator; Stein 1973).
7. All agar treatments should be autoclaved to ensure sterility. (Does this affect the vitamin treatment?)
8. The chamber is sealed with a coarse, porous porcelain or fused silica (2.6-cm diameter) disk (crucible cover) (Leco Corp. #528-041) that has been cleaned by soaking in a 10% HCl for 48 hr and rinsing copiously with distilled water.
9. Heat each disc on a hot plate. Seal the agar-filled vial by placing the hot disc on top of the vial, melting the plastic at the mouth of the vial, and molding it around the disc.
10. Turn the vial upside-down, allowing the agar mixture to solidify in contact with the porous disc.
11. Cap and color-code finished vials according to the treatment they contain.
12. Arrange the vials in batches of 42; each batch will contain 6 replicates of each of the seven treatments.
13. Place each batch in a wooden holder(s), which are strips of lumber with pre-drilled holes (3-cm diameter) and mounted on 1.2 m x 0.31 m

plywood. Secure the vials into the holes with a small spot of silicon sealant on the bottom of each vial (use as little as possible to avoid the effects of acetic acid leaching from the sealant).

a. At each site in the stream, secure the wooden holder to the stream bottom. Use two restraints: a rope running between opposite shores and looped through a hole on the upstream side of the wooden base; and two metal spikes at each end of the board, driven through the base and into the rocky bottom, with flat rocks placed over the stakes at each end. This should ensure that the boards will remain stationary on the river bottom even in high flow periods.

b. Leave the boards and the agar vials undisturbed for three weeks. This procedure will be done three times, with one week of overlap between experiments.

c. When an incubation period is over, unfasten the board from the river bottom but keep it submerged. Carefully maneuver it to the shore.

d. Remove the vials one at a time by color code and place the discs into pre-labeled plastic 125-ml urine cups. The discs can be removed by gently squeezing the mouth of the vial with pliers.

e. Three discs from each treatment can be placed in the cups dry; they will be assayed for chlorophyll α biomass. The other three should be placed in cups with about 50 ml of water and remain completely submerged; they will be assayed for primary productivity.

f. Return the samples to the lab and give them to Miller's group.

D. Ammonium – Phenol method

1. This method was used 1983 -1999.

2. Ammonium Manual Chemistry

   a. In order to detect the low levels of ammonium found in most unfertilized stream reaches and lakes, it is necessary to use a manual method at Toolik. A blue compound, indolphenol blue, is formed by the reaction of ammonium, hypochlorite, and phenol. The color is intensified by nitroprusside. A spectrophotometer @ 630 nm with a light path capacity of 5 cm is used.

   b. The protocol below was adapted by Bowden and Finlay from the automated phenate method described in section 4500 H of Standard Methods (1985).

3. General Comments
   - **a.** Wear gloves at all points of this procedure because it is easy to introduce contamination from bare hands, and the reagents are toxic.
   - **b.** Always use ammonium-free water for all reagents. Get the water from the nanopure unit just before you use it.
   - **c.** Acid wash all glass and plasticware (including volumetric flasks and pipettes) in the acid bath dedicated for ammonium chemistry (to avoid contamination from other sources). Following the acid bath, immediately rinse 3-5 times with fresh distilled water.
   - **d.** Phenol breaks down plastic so use glass to store this reagent.
   - **e.** Use dedicated glassware for each step of the procedure, including the same containers for the same standards (especially blanks). Do not use glassware that has been used for nitrate chemistry, which uses ammonium as a reagent.
   - **f.** Because the reagents are toxic, place all bottles in a wash basin in case of spill, and work in a fume hood if available.
   - **g.** Use repipetters for dispensing reagents into the sample. This speeds things up considerably. There are 500-ml amber bottles for this purpose. Use of repipetters also minimizes possible contamination.
   - **h.** When making the standard dilution series, make sure the pipetters are calibrated properly. It is important to get very accurate dilutions when working with such low concentrations. Alternatively, you can use volumetric pipettes for most of the dilution series.
   - **i.** Make sure waste and old reagents are stored in the properly labeled container, including the waste that runs through the spec.
   - **j.** Whenever possible, use DI direct from the nanopure unit, without transporting in an additional container.

4. Reagent preparation
   - **a.** Hypochlorous acid 5% NaOCl (Bleach)
     - Prepare fresh for each day. 100 ml of reagent is enough for about 100 samples and standards.
     - Add 20 ml 5% NaOCl (chlorox) to a 100-ml volumetric flask.
     - Add 80 ml DI water to 100-ml mark.
     - Adjust pH to 6.5-7.0 with 1 N HCl. Pour a little of prepared solution into bottle cap or petri dish and measure with pH paper. Add a couple of ml of HCl to reagent bottle and recheck. Repeat until color of pH paper is correct. The acidity change will be rapid, so do not add too much acid at any given time or you'll have to start over.
     - Chlorox should be brought up fresh each year, or perhaps several times during the summer. It should be stored in the refrigerator when not used. Caution: a large head space allows chlorox to oxidate, increasing the pH.
   - **b.** Phenate (Phenol, water, NaOH)

- Prepare fresh for each day. Refrigerate in amber glass container if saving overnight.
- *Handle with care, read MSDS for Phenol prior to use*
- To a 100-ml volumetric flask, add 9.3 ml of liquid phenol (the liquid form is easier to use than the crystal form) using a volumetric pipette with a pipette bulb.
- Bring to 100 ml mark with fresh DI water.
- While cooling with tap water (on ice if necessary) slowly add 3.2 g NaOH and dissolve. The addition of NaOH is an exothermic reaction, so do not stopper the flask. Excess NaOH will speed color development but will cause a urine yellow color to develop in all samples. Yellow color may affect blank absorbance, so try to avoid.

c. Nitroprusside (sodium nitroferricyanide)

- Store in amber glass container for up to 1 month.
- *Handle with care, read MSDS for nitroprusside prior to use*
- Add 800 ml DI water to a 1-L volumetric flask.
- Add 3.5 g sodium nitroprusside and swirl until dissolved.
- Fill to 1 liter with DI water.

5. Standard Preparation

a. Stock A solution (prepare at the beginning of the summer)

- Acid wash 1-L volumetric flask
- Make 1 mM solution of NH_4-N from $(NH_4)_2SO_4$
- MW of $(NH_4)_2SO_4$ = 132.14
- 2 moles of N per mole of ammonium sulfate
- 1 M NH_4-N = 66.07 g of ammonium sulfate in 1 L of DI
- 1 mM NH_4-N = 0.06607 g in 1 L of water. Make a note if you end up using a slightly different amount and adjust concentration when making up standard curve.
- Use ammonium sulfate that has been dried (in a glass vial) in the drying oven for 12 hours before using.
- Store solution in labeled amber container in the refrigerator

b. Stock B - Secondary stock solution (prepare just prior to use)

- Acid wash the 0.5-L volumetric flask and rinse several times with DI
- To prereact the flask, add 20 ml of DI water, 1.2 ml phenate, 1.0 ml hypochlorous acid, and 0.8 ml nitroprusside and let sit for several hours. Swirl to clean inside. Dump reagent in waste container and rinse with DI 3-5 times.
- Using a calibrated pipetter, or volumetric pipettes, make a 10-□mole secondary stock to use for preparing the dilution series
  - To a 0.5-L volumetric flask, add 5 ml Stock A, and fill to the 0.5-L mark with fresh DI
  - Invert several times.

c. Dilution Series

- For low levels, use 4-5 standards with concentrations less than 1 □Mol.
- Use dedicated 100-ml volumetric flasks that have been acid washed and prereacted (as described for stock B preparation).
- Series should be made just prior to use, because low standards in particular absorb ammonium very easily. DI water should be fresh from the nanopure filter unit.
- If using pipetter, make sure each volume is calibrated.
- Try to use one type of volumetric flask for all dilutions to avoid dilution errors.
- Recommended series:
  - 0.05 □mol .5ml of stock B, 99.5 ml fresh DI
  - 0.1 1ml 99
  - 0.2 2ml 98
  - 0.4 4ml 96
  - 0.6 6ml 94
  - 1.0 10ml 90
  - 5.0 50ml 50

6. remaining stock B solution
   - blank 100ml DI water
7. Get the dedicated 50-ml centrifuge tubes still containing reagent from the last run, shake, and dump reagent into the proper waste container. Rinse 3-5 times with fresh DI water.
8. Pour 20 ml of standard from volumetric flask to 50-ml centrifuge tubes dedicated for that standard concentration. Volume does not have to be exactly 20 ml because the reagents are added in excess.
9. Use 2 tubes for each standard and 4 tubes for the blank. In 1994, 2 of the blanks received superadditions of reagents. To these, 1.5 times the normal amount of reagents was added. This was helpful in determining possible sources of contamination. Absorbance should be equal for both types of blanks and should be less than the lowest standard.
10. Color intensity is affected by age of reagents. Thus, reagent blanks have to be run with each set of samples and standards.
11. Procedure for sample ammonium determination
    a. Reagents should be left in sample tube from previous run. Prior to going into field, shake reagent to prereact the entire inside surface, then dump reagent into ammonium chemistry waste container and rinse several times with fresh DI. This should be done for standards and blanks as well. Shake dry the tubes.
    b. Return from the field with 20 ml of filtered water in each centrifuge tube from each station.
    - Add 1.2 ml phenate reagent and swirl.
    - Add 1.0 ml hypochlorous acid reagent and swirl.
    - Add 0.8 ml nitroprusside reagent and swirl.

- There should now be about 23 ml total in each sample tube.
- At this time, you should also add the three reagents, as described above, to the standard dilution series and to the blanks; remember to add 1.5x the normal reagent amount to two of the four blanks.
- Cap tightly and place samples in dark for at least 1 hour but no more than 24 hours. (Color formation should be complete after 10 min).

12. Reading on the Spectrophotometer (Terrestrial Trailer)
    - **a.** Samples should be read immediately after reading the standards, or else they may develop more intense color and they will no longer be comparable to the standards.
    - **b.** Make sure Mr. Sipper is set to sip 5ml?
    - **c.** Set wavelength to 630 nm and make sure the spec is reading absorbance.
    - **d.** Wear gloves when reading standards and samples on the spec. Ammonium from hands could be left on sipper tube, and reagents are harmful.
    - **e.** Make sure the waste tube enters the ammonium waste container.
    - **f.** Sip DI water and zero the reading.
    - **g.** Sip standards so that low standards are read first (start with blanks and work way up) After each standard, sip water through and rezero if necessary. Sip DI between high standard and first sample to remove any residual NH_4.
    - **h.** Sip again to make sure all residual material is removed and the reading is stable. Rezero the spec.
    - **i.** Sip your samples through and record the values in a notebook. After reading the entire sample series once, read them each a second time. Make sure you avoid sipping air into the spec, as this might interfere with your readings.
    - **j.** Between samples, wipe the sipper tube dry with a kimwipe so that contamination is minimized.
    - **k.** If a sample absorbance is higher than highest standards, you will need to dilute the sample. (can you do this in the tube?)

13. Regression
    - **a.** Subtract the average absorbance of the blanks from all standard readings. Then regress the concentrations of the standards against their blank-corrected absorbances. You should get an r^2 of at least three nines (e.g., r^2=.9992).
    - **b.** Use the regression equation to calculate the concentration of ammonium in the samples.

14. Notes
    - **a.** We have found it better to leave reagent in sample and standard tubes and flasks from the last run until right before the next run.

Just before run, dump reagent in waste container and rinse tubes with fresh DI.

b. Standards should be made fresh just prior to run. Low standards that have sat all day tend to absorb ammonium. The same holds true for DI water. Always get fresh water from nanopure filtration unit.

15. Checklist

a. Beginning of summer
- Make ammonium sulfate stock solution
- Make nitroprusside reagent
- Make acid bath (change monthly?)

b. Day before sampling
- Make phenate reagent
- Make Hypochlorous reagent
- acid wash volumetric flasks and pipettes (wash flasks only before first sampling; afterwards, leave reagents in flasks and prereact).
- rinse volumetric flasks with DI
- Prereact all volumetric flasks (add fresh reagent each time).
- Prereact 50-ml centrifuge tubes (both standards and sample tubes). This is a separate step only for the first sample time. After the first sampling old reagent will be left in the tubes and used to prereact.

c. Before sampling
- Shake sample tubes
- Dump reagent in proper waste container
- Rinse several times with DI
- Shake dry
- Recap
- Rinse in field with filtered water

d. After sampling
- Place samples in refrigerator while preparing everything
- Shake prereacting volumetric flasks
- Dump reagents in proper waste container
- Rinse several times with DI
- Make stock standard B (10 □mol/L in 0.5-L volumetric)
- Calibrate pipetters (if using these)
- Make dilutions. Use the same flask for each standard each week
- Shake prereacting standard tubes
- Dump reagent
- Rinse several times with DI
- Pour standard in proper tube dedicated to that standard (as clos e to 20 ml as possible)

- Add reagents from repipetters to standard and sample tubes
- Wait an hour or two.
- Make sure spec waste is hooked up to right container
- Read on spec in order that reagents were added.
- Rinse spec w/ di to clean out all reagents
- Leave reagents in the flask

E. Nitrate – Lachat Method used at the MBL.

1. This Lachat method (QuikChem (Method 31-107-04-1C) was used for Nitrate analysis at the MBL lab. Method 31-107-04-1E is now used at the RESL
2. Standards for this method are KNO3, opposed to NaKO3 and KNO2, opposed to KNO2, which is used in the more current method.

Nitrates samples were analyzed on a Lachat Quik-Chem 8000, following Quik-Chem Method 31-107-04-1-C.

2000. Diamond, David H. Determination of nitrate and/or nitrite in brackish or seawater by flow injection analysis colorimetry. Quik-Chem Method 31-107-04-1-C. Zellweger Ananlytics, Lachat Instrument Division, 6645 West Mill Road, Milwaukee, WI 53218.

Summary: Samples are passed through a copperized cadmium column to reduce nitrate to nitrite. The nitrite (both original and reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-napthyl)-ethylenediamine dihydrochoride to form a highly colored azo dye which is measured colorimetrically.

Working Range: 5-50µm, MDL 0.12µm

Standard Preparation:
50mM stock standard-in a 1 liter volumetric flask dissolve 0.5055g dried (1hour at 60°C) potassium nitrate (KNO3) into about 800ml distilled water. Dilute to 1 liter with DI water and invert several times to mix. Can be stored refrigerated for up to 3 months.
50µm Working Stock Standard-In 1 L volumetric dilute 10ml 50mM stock to mark with DI water, invert to mix.

Working Standards (Prepare Daily):
50µm-dilute 250ml 50µm working stock std. with 0ml DI water
25µm-dilute 125ml 50µm working stock std. with 125ml DI water
10µm-dilute 50ml 50µm working stock std. with 200ml DI water
5µm-dilute 25ml 50µm working stock std. with 225ml DI water

II. 0µm-250ml DI water

V. Appendix B – Locations of Equipment

A. At Toolik

1. Wet Lab

a. Streams Desk

- Office supplies
- Labels
- Filters, forceps
- Wader patches
- Truck keys and info
- Protocols
- Lab books

b. Streams Shelves - Regularly used field gear

- Nuts and scrubs kits
- Yoy containers and nets
- Wading rods
- Flowtracker
- Batteries
- Label tape
- Ziplock bags
- Caulk gun

c. Streams Closet

- Waders
- Wading boots
- Bug shirts
- Head nets

d. Fluorometer room – dark room

- Ammonium fluorometer
- Ammonium scint vial flats
- OPA reagent and buffer
- Chlorophyll fluorometer
- Borosilicate tubes
- Cooler for Chlorophyll samples
- Acetone

e. Spec room

- Cary spec set up for phosphorus analysis
- Phosphorus working reagent
- Balance
- Vortex

2. Wet Lab Conex
   a. Sample bottles, petri dishes, etc…
   b. Wash bottles
   c. Amber liter bottles
   d. Centrifuge tubes
   e. Scint vials
   f. Dripper parts and pumps
   g. Old field equipment bits
3. Lab 3 – LTER things
   a. YOY balance
   b. YOY knockout solutions
   c. Fishing rods and reels
   d. Waders
   e. Wading boots
   f. Electroshocker
   g. Oksrukuyik stream level datalogger

B. At the RESL - 2013

1. RESL Lab
   a. Chemicals for reagents, standards
      - Ascorbic acid
      - Sulfanilamide
      - Potassium persulfate
      - Potassium nitrate

   b. Filter samples
   c. Algal comp samples
   d. Test tubes
      - TDP/PP with polyphenol screw top caps in racks – 15mm and 30mm
      - TDN/DOC with septa screw top caps – ash before analysis
      - NO3 borosilicate tubes in racks
   e. Filters – 47mm and 25mm – some ashed
   f. Standard volumetric flasks, other standard glassware.

2. General Use Lab
   a. Sodium hydroxide 15N – base cabinet
   b. Phosphoric acid – 85% - acid cabinet
   c. Sulfuric Acid – 36N – acid cabinet
   d. Hydrochloric Acid 12N – acid cabinet

C. Stored at the MBL

1. Walk in Cooler
    - **a.** Water samples from previous summers
        - TDP - acidified
        - TDN/DOC - acidified
        - Anions
        - Cations - acidified
        - Alkalinity
2. Walk in Freezer
    - **a.** Frozen samples from previous summers
        - NO3
3. Office
    - **a.** Old field notebooks
    - **b.** State of the river reports
    - **c.** Old reprints
    - **d.** Data backup disks
4. Long term storage and memorial circle
    - **a.** Old particulate filters