Master Protocol

Yukon River Synoptics

Field Operations

Hydrolab:

Calibrated before each sampling
Hang from the side of the workboat
Record data on AK District Field note sheet

IRGA Measurements (all sites except main stem grabs):

1. Turn on IRGA by pressing ON/OFF button. Note: when pressing buttons, press them for about 1 second. Also note that when you push buttons, there is often a short delay before the IRGA reacts and/or your numbers appear on the display, so don't keep pushing buttons if there isn't a nanosecond response. Also note that the IRGA display might not work in extreme cold. Keeping the IRGA inside your jacket may help.
2. IMPORTANT: NEVER LET WATER GET SUCKED INTO THE IRGA. IT WILL DIE. Use a syringe filter on the inlet port of the IRGA to keep water out, and change the filter often if it gets wet.
3. Let it warm up for 10-15 minutes.
4. Calibrate: The IRGA should be calibrated each morning before leaving for the field. Mark and Doug will be the primary person for calibrating and running the IRGA. As a check on the calibration, periodically write down the atmospheric air CO2 concentration by holding the IRGA in a clean air stream (away from gas-powered ice augers, running snow mobiles, outboard exhaust, heavy breathers, etc.). Last summer, our air readings averaged 365 ppm (with a general range from 360-370 ppm). If the IRGA is already calibrated, skip this step. Just warm up the IRGA and proceed with your work (step 5).
    a. Hit "N" to go to main menu
    b. Hit "3" to go to "Cal"
    c. Enter standard concentration. Note that display will read "?????" Five digits will have to be entered, i.e., if the standard concentration is 350 ppm, enter "00350"
    d. Display will ask you to choose Dynamic or Static mode. Hit "1" for Dynamic mode.
    e. Display will read, "When display is steady, press 0."
    f. Hook up balloon of standard gas to the inlet port on the back of the IRGA
    g. When display is steady, hit "0"
    h. Display will read "Calculating scaling factor," then "Calibration Complete"
    i. Display will return to menu. To return to readings, press "1" for Record, then "Y" for YES.
5. Once calibrated, (and you're on site), hold the IRGA up in the air away from possible contamination sources, and record the air CO2.
6. Rinse a 60 ml polypro syringe, then fill with bubble-free water from 10 cm below the water surface. Knock any bubbles out of the syringe. Push the plunger to expel about 30 mls of water, so that you are left with **EXACTLY** 30 mls of water in the syringe. Fill the syringe **EXACTLY** to the 60 ml mark with **CLEAN** air by pulling on the plunger. Make sure to note the water temp.
7. Once you have 30 mls of water and 30 mls of gas, close the stopcock and shake the syringe for two minutes to equilibrate.
8. The IRGA can operate in two modes, dynamic and static. In dynamic mode, an internal pump constantly draws air through the inlet port on the back, and gives you a constant readout. In static mode, the internal pump is not on, and the machine does not see any sample unless you inject it manually. Dynamic mode is what you will want when you are calibrating, or taking a reading of the clean air. Static mode is what you will want when you equilibrate a syringe of water and inject the gas into the machine.
9. To switch from dynamic to static mode, first press "N" to get to the main menu. Hit "1" for Record Menu. You'll see that option "4" will show you whether the IRGA is currently in dynamic or static mode. By pressing "4," you toggle between the two modes. After pressing "4" it will ask

you to hit "Y" to confirm that you want to change the mode. Then the display will read, "wait until zero over and pump off." When you hear the pump go off, you can inject your equilibrated air sample.

10. Take your 60 ml syringe of equilibrated water and gas, and hold it upright. Tap the syringe, to try to get all water down from the stopcock into the syringe.
11. Take a dry 30 ml nylon syringe with stopcock, and attach it to the stopcock on the 60 ml syringe of equilibrated water and gas. Open the stopcock on the 60 ml syringe (being careful not to lose the gas to the atmosphere), and push on the plunger, transferring GAS ONLY to the nylon syringe. Close off the stopcock on the nylon syringe, again being careful not to lose gas to the atmosphere. It is sometimes difficult to transfer just gas, without getting a small drop of water in the nylon syringe. It just can't be helped. As long as you inject this sample fairly quickly, and you do not inject that drop of water into the IRGA, things will work.
12. Make sure there's a small syringe filter on the IRGA injection port in the back, and attach the nylon syringe to the filter. Open the stopcock, and slowly and steadily inject the gas sample into the IRGA. Do this while watching the display. Numbers will jump around a bit, but should remain fairly stable by the time you finish injecting all the gas. Record the value.
13. At each site, you will want to inject no more than 5 of these injections. If you can get three injections to read within 10% of each other, that's enough. But 5 injections would be the max for any site.
14. Switch nylon syringes every time, so you're using dry ones.
15. After finished with work, change the IRGA from static back to dynamic mode, and take an air reading. At the end of the day you'll want to also take a reading of a calibration standard.
16. If continuing with chamber work, see instructions below. Otherwise, turn off the IRGA by pressing both the ON/OFF and OFF/L buttons at the same time.

Chamber Work (at every site except main stem grabs):

1. Make sure IRGA has warmed up.
2. Make sure IRGA is reading in dynamic mode (see instructions above).
3. Hook up tubing between IRGA and chamber.
4. Change the IRGA zero interval from 2 minutes (default) to 30 minutes:
    j. Hit N to go to main menu.
    k. Hit 2 (Set)
    l. Hit 1 (ZeroT)
    m. Enter 30
    n. To return to constant readout, hit 1 (Rec), then Y.
5. When IRGA reading is steady, place chamber over the side of the boat and onto the water surface. This will require steadying the chamber so it does not swim in the current or float away.
6. The other person records the IRGA reading every 30 seconds for five minutes.
7. After five minutes, pull the chamber from the water, and wait for IRGA readings to stabilize at atmospheric levels.
8. Repeat exercise two more times.
9. If using the bucket chamber, measure the height between the water surface and the top of the bucket while it is floating on the water.
10. Turn off IRGA when done (press both the ON/OFF and OFF/L buttons at the same time.

Secchi disc

Drop in water column and measure depth at which whit and black is indistinguishable

Discharge:

Measure Discharge (ADCP) at each sampling location

Main stem and large tribs: establish 5 EDI verticals along transect using ADCP (600 or 1200 kHz) and DGPS

When ADCP cannot be used:

Smaller tribs (non-wading): use Boat Price meter Kit

Smaller tribs (wading): use Wading Price meter Kit

If pressed for time and ADCP or Price meter not possible: take depth-sounding profile and record a centroid velocity with Price meter

EDI Sampling:

D-96: 3-L Teflon Sampler bags

Main Stem sampling (requires power unit)

Be sure bag is secure in D-96 sampler with Velcro strap

Be sure nozzle is oriented correctly in nozzle holder

One full rinse with sample water

Be sure all sediment is washed from bag

Be sure to homogenize each bag sample to ensure all sediment is transported to the sample vessel

D-96A (lighter version): 3-L Teflon Sampler bags

Tribs with lower flow (2-6 ft/sec)(if trib is shallow, may require hand cranking)

Be sure bag is secure in D-96 sampler with Velcro strap

Be sure nozzle is oriented correctly in nozzle holder

One full rinse with sample water

Be sure all sediment is washed from bag

DH-77 (hand-held): 1-L bottle

Wadable tribs

EDI verticals: Transit time calculated from Discharge data

Station 1: First vertical: At designated sites, collect Sed Chem sample “Art bottles”; pour directly into 10-L jug

Second vertical: pour into jerican (total volume = 15 L)

Station 2-5: repeat

If EDI not possible; take a centroid grab samples

Samples: Never Come Back Without Samples

QA/QC:

Blanks

Fill a churn with FBX DI water and process in the field (beginning and end of trip)

Duplicate

Yukon River @ Whitehorse (if no time, make every attempt to collect another DUP at a more opportune time

Jerican: 20-L: transport vessel to truck lab when sample transport is not possible with churn

Rinse Jerican with sample water
Fill by EDI into jerican
Screw on lid very tight
Secure jerican inside a cooler and ice up ASAP if possible
Return full jerican (15 L) to truck lab

Main Stem Grabs (formerly known as "Reverse Deadhead")

~8 Main stem grab samples collected by workboat passenger while enroute to sites along Yukon River. Collected downstream of trib confluences past mixing zones. Use Field sheet to record data
Samples collected:

DOC (40 ml glass amber vial) filtered
Majors (filtered with Acrodisc/syringe, 125 ml black cap FU, need min of 30 mls)
CO2 (serum bottles) (see "Striegl: Gas Sampling" for procedure)

Aiken: DOC Characterization (formerly known as "cubies")

Isolation samples: Grab samples collected in four 10-L poly bottles (same as "Art bottles" at selected sites. Total sample volume: 40 L. Use the HDPE , LDPE or acrylic pitcher to pour sample water into bottles. Screw on lids tightly. Label with site name, date, time. Keep chilled with ice, if possible. Secure in cooler with strapping tape. Return samples to truck lab for filtering.

Barber: POP

Grab sample from effluent stream just below lagoons outside Whitehorse. Two 1-L amber glass bottles, RU (Raw, unfiltered). Keep chilled and dark.

Striegl: Gas Sampling

All Gas Samples Must Be Filtered

1. Record Secchi depth.
2. Rinse 60 ml polypro syringe 2x with water
3. Carefully fill the syringe with **bubble-free** sample water from 5-10 cm below the water surface.
4. Attach the stacked syringe filters to the syringe (the 1.6 uM filter attaches to the syringe tip itself, and the 0.45 uM filter attaches to the 1.6 uM filter). If you have the single 0.45 uM 25mm filter, use that instead of the stacked filters. Make sure connections are tight.
5. Attach the needle to the tip of the 0.45 uM filter. Make sure connection is tight.

6. While pointing the syringe up, push on the plunger to expel air from the filter(s) and needle.
7. Inject 15 ml of sample water into each serum bottle through the stacked filters. *IMPORTANT*:
    a. While injecting sample water into the bottles, it is best to have the syringe pointed up. This way, if pressure on the syringe plunger is accidentally lost, and gas escapes from the bottle into the syringe, it is easy to quickly push the escaped gas back into the serum bottle. Also, when removing the needle from the serum stopper, having the serum bottle upside down on top of the syringe needle means that gas cannot follow the needle as it is removed and escape to the atmosphere.
    b. While injecting sample, please watch the filter and needle connections closely to make sure the seals are not leaking.
8. It is ok to switch filters if they become too clogged to push sample through. If new filters are put on the syringe, remember to expel the air from those filters before injecting sample into the serum bottle. Also please note in the field notes how many sets of filters were used.
9. It is also ok (and recommended) to switch needles. Normally I switch needles after injecting water into two bottles. The more times you push a needle through the serum stopper, the more it sticks, increasing the risk of pulling the syringe off the needle and leaving the needle in the stopper, in which case you lose the gas to the atmosphere.
10. Record the water temp on each bottle, along with site/date/time.

Striegl/Kendall: 13C-DIC and 13C-DOC Sampling:

1. Rinse 60 ml polypro syringe 2x with water.
2. Carefully fill the syringe with **bubble-free** sample water from 5-10 cm below the water surface.
3. Attach 0.45uM filter (or stacked filters) to syringe, as described above.
4. Filter water into the clear 20ml vial that contains CuSO4. Fill the bottle completely, until there is a positive meniscus on the top of the vial (i.e., the water is slightly overflowing the top of the vial). Making sure there are no bubbles in the vial, and no headspace, screw the cap down tight. This sample is for 13C-DIC.
5. Repeat the procedure with the 20ml amber vial. This sample is for 13C-DOC.
6. Label bottles with site/date/time and "13C-DIC" or "13C-DOC".
7. Store chilled and dark.

Horowitz: Sediment-metals

MAIN STEM AND MAJOR TRIBS ONLY (see itinerary)
See EDI sampling above.
Total "Art bottle*" volume: 15 L

Keep Art bottles in the cooler and open the cooler and jugs ONLY when pouring in the sample. This keeps dust contamination to a minimum. Each bottle is double bagged as an extra layer of defense. Each sample takes 2 bottles, but one is only half full (5 verticals x 3L/vertical = 15L). Secure bottle caps firmly and tape if necessary. **Don't ever store them on their sides or upside down**. Label each jug with site name, date and time. Store at air temp.

NOTE: 2 jugs fit loosely in a standard cooler or 3 fit tight in a wide cooler. A 4-jug cooler will be 85 lbs; very heavy but would probably be the most space efficient.

* "Art Bottles" are 10-L poly jugs

Krabbenhoft: Mercury

Grab samples to be collected at the centroid of flow.
Clean hands/dirty hands protocol are used to remove 1 liter Teflon bottle from bags and dip sample from bow of boat while holding station or moving slightly into flow. Rinse bottle minimum of three times before collection of sample. Once sample has been collected, replace cap tightly, return to bags, and record Site, Date and Time on outer bag. Place bagged sample into cooler in the dark and on ice if possible. Using clean hands/dirty hands protocol collect bottom sediment sample near shore within transect. Remove vial from bags and scoop the upper 2-4 cm of sediment from bottom. Tightly cap vial, return to bags, and label bag with Site, Date, and Time. Place sample in cooler (freeze as soon as possible.

Raymond: 14C-DIC

These samples can be taken off the boat with the other DIC samples. I have enclosed a 60ml syringe and sent a bunch of disposable filter discs separately to Zan from Fisher. Give the syringe a rinse or two. Fill it with water, put the syringe filter on the syringe and the tubing on the syringe filter. Put the tubing at the bottom of one of the 60ml amber bottles. Filter the water into the bottle. Take syringe off, refill and try to get another 60ml through, allowing the water to overfill- while filling from the bottom. Acidify with 0.5ml of the phosphoric (this can be done back in the labtruck when possible), screw the top down tight and place black electrical tape around the seal of the cap

Kendall/Finlay: Organic Matter/Food Web Sampling

Plan for 5 sites for samples described below, and will pack extra bottles for opportunistic samples (e.g. fish). Samples will be collected from tributary streams that can be accessed safely. Specific sites will be decided upon by the crews in the field. Ideally, sites will span a range of stream sizes, and all sites will be revisited during the August 2003 trip. Parentheses indicate interested parties.

Benthic sediments/organic matter (Carol, Jacques) At each site, 3 grab samples of surficial benthic organic matter will be scooped up from different locations in the main channel using a dipper attached to a pole(?). Samples will be transferred to small whirlpak bags or zip locks and kept cool until they are frozen on the boat. Materials: scooper, plastic bags.

Riparian vegetation (Carol, Jacques, George) At each site, foliage of 2 dominant species (e.g. black spruce and willow) will be collected by compositing foliar material from several trees into a small paper bag or coin envelope. Samples can be air dried on the boat or frozen. Materials: coin envelopes and small paper bags.

Food webs (Carol, Jacques) At each site, attached algae, benthic invertebrates, and fish will be collected as available. In mid June there may not be much to choose from, so if field crews note presence of algae/bugs/fish, go for it! Ideally algal and invertebrate samples would be composited for the same species into small glass vials from several areas at the site. Samples should be kept cool until they are frozen on the boat. For each fish species (e.g. grayling, burbot, pike, whitefish), two to three individuals per site would be ideal. After recording lengths and weights, a small amount of dorsal muscle must be dissected out without skin, placed into a glass vial, and preserve with ethanol. Materials: 7ml glass vials, kick net, fishing gear, ruler, field balance, forceps.

Shiller: Trace Metals

Initial Advice

1. This protocol is designed to be fairly straight forward. One of the most important things for you to keep in mind at all times is that it's easy to contaminate trace element samples if you're careless, use the wrong materials, or expose the sample for too long. Fortunately, with just a little care, it's easy to obtain a clean sample. Please wear polyethylene gloves at all times. Change your gloves when they become dirty. Ideally, it's best to work with two people: a "clean hands" person who collects and processes the sample and a "dirty hands" person who opens ziplock bags and hands bottles to the clean hands person. Both people should wear poly gloves.

2. Please filter the water as soon as possible after sample collection—ideally, immediately after collection in the field because microbial and adsorption processes can change the dissolved/ particulate partitioning. If storage is necessary, store Hold bottles in double ziplock bags, chilled and in the dark. You will be collecting two bottles of unfiltered water (replicate samples) and from each bottle you will then filter one aliquot through a 0.45 :m filter and another aliquot through a 0.02 :m filter.

Collecting the Sample

3. It is assumed that you will be collecting samples from small workboats where one can easily collect a surface grab sample by hand. Wear polyethylene gloves. Make sure you sample from the bow of the boat while headed slowly into the current (have boat go at a speed such that there is a decent flow of water past the boat but not so fast that the bow is splashing up water). Carefully immerse pre-cleaned 250 or 500 ml bottle in stream, fill and rinse a couple of times (including the cap). Repeat with a second bottle so as to collect two replicate samples. (If using the "pooper scooper" or other clean bottle holder, see separate instructions below.)

Sample Filtration

4. You can use polyethylene gloves or sheeting to make a reasonably clean work surface. Caps of samples bottles you are filling can be placed on or inside a clean glove.

5. Rinse pre-cleaned syringe with sample. (Note: there are two ways to fill the syringe. I usually pull out the plunger, put the filter on the end, and then pour water into the open syringe. You can also use the syringe itself to suck up water and then place the filter on the end. If you opt for sticking the syringe into the sample: Make sure the outside of the syringe has not gotten dirty!)

6. Slowly push 15 ml of sample through a pre-cleaned Whatman Puradisc PP 0.45 :m syringe filter to rinse (don't collect this water, discard it). Use another 5 ml of filtrate to rinse a pre-cleaned 15 ml sample bottle. Then, push ~15 ml of sample through syringe and into the 15 ml sample bottle (i.e., fill bottle to where it starts to narrow). Tightly close 15 ml bottle and store. In this procedure, be careful of drips: you don't want to get a drip of contaminated or unfiltered water in the 15 ml sample bottle.

7. Filter a sample using a pre-cleaned Anotop 0.02 :m syringe filter using the following procedure. You can use the same syringe as in step 6. Anotop filters have a very small pore size and filter far more slowly than the Puradisc PP's; therefore, while it is possible to use hand pressure on the syringe to push water through the filter, you will find it much easier to use the nylon syringe press and stand. However, care must be exercised with this press—if you crank down too tightly it is possible to burst the filter! You will find a Puradisc PP connected on top of an Anotop: this filter stack allows the Puradisc to operate as a prefilter and aids the filtering process. Connect the syringe to the Puradisc/Anotop filter stack and put about 45 ml of unfiltered water into the syringe. Place syringe/filter assembly in the nylon press and tighten upper thumbscrews so that the syringe plunger is about 3-5 mL above the water surface in the syringe barrel. Place the press on the stand and let at least 10 mL of water flow through the filter stack to rinse it---this is very important (we'll provide some additional clean small bottles to use for collecting this "waste" rinse water). Use the next ~5 ml of filtered water to rinse a precleaned 15 ml sample bottle. Finally, collect ~15 mL of filtered water in the cleaned, rinsed 15 mL sample bottle. This is a somewhat (!)

tedious procedure because filtered water will come out of the Anotop at a rate of only about a drop/sec. There is no way to rush the procedure---just keep constant pressure on the syringe. In general, don't tighten the nylon thumbscrews of the press more than 10 turns at a time. Every few minutes you can tighten the thumbscrews as the water level in the syringe moves down. (A rule of thumb here is to watch the plunger and turn the thumb screws to keep it about the same distance above the water surface in the syringe barrel—you'll find that turning both thumb screws about 2 turns will move the plunger a 1 mL down the barrel.) We are providing two nylon presses, so you can process both replicates at the same time.

8. Repeat steps 6 & 7 for the other replicate sample.

Finale

9. Screw on the caps of the 15 mL sample bottle tightly! Store samples in double ziplock bags. Keep them cool and in the dark. We will acidify the samples in our lab at a later date. Record pertinent data (sample location and time as well as other pertinent notes). You can discard used syringes, filters, and sample collection bottles (note: Nalgene polyethylene bottles can be given to a recycler.)

Sampling Protocol For Genetic Studies Of Canada Geese

Important! State and Federal U.S. Fish and Wildlife Permits are necessary for collection of the materials listed below. If you plan to collect materials, please contact John Pearce at the address below so that we can add your name to our permit.

Sample Sizes

Ideally, we would like to obtain 20 samples per location of known breeding birds. However, we realize that Canada Geese are widely dispersed and nesting in low densities in the proposed sampling areas. Therefore, whatever numbers you are able to obtain will be of value. Samples from spring or fall migrants are of interest, but are of secondary priority.

Collection Information

With all sampling, material should be clearly labeled with the following information:

- Date of collection.
- Exact location - Name of river, number of miles to nearest village, or GPS position if possible.
- Your name and contact phone number and address.
- Status of the bird - Was the bird an adult or juvenile, nesting, molting, found dead, shot etc.

Types Of Materials

The following is a list of materials that can be sampled for DNA. Collect whatever is easiest and the most logistically feasible. For nests that are found, we would prefer a sample of a developing egg (see below). Nest feathers and eggshell membranes would be the next best tissue type to collect.

Measurement data is also of interest for morphological comparisons. If you plan to collect adult birds or capture nesting or molting adults, please contact John Pearce so we can discuss options for collecting measurement data.

Sampling Instructions

Nest materials:

1) Feathers. Collect as many feathers from the nest as you can find. Do not collect down as this has no recoverable DNA. Feathers should only be collected from inside the nest, not from out on the tundra. Feathers from each nest should be stored at room temperature in a paper envelope.
2) Eggs. Do not collect fresh or undeveloped eggs because they have no recoverable DNA. Eggs must float in water or have visible embryonic development when held up to the light.

    a. Collect one egg per nest.
    b. Cover the remaining eggs with down and walk away from the nest.
    c. Measure the length and width of the egg using calipers.
    d. Crack the egg open. Sample some of the bloody, veinous material with tweezers and place the sample into a tube of tissue preservation buffer.
    e. Label the tube with date, location, etc. The tube can be stored at room temperature until you are ready to mail it back to us.
    f. Discard the broken egg away from the nest.
    g. Wash the tweezers after each egg to avoid cross-contamination.

3) Egg shell membranes. After hatch, eggshells and membranes (attached to the inside of the shells) may be present in the nest. Shells have no recoverable DNA, only the membranes. Collect one membrane and place it into a large paper envelope. Write collection information on the envelope.

Birds:

1) Blood or tissue. Captured birds can be sampled for blood, but only bleed birds if you are trained in this procedure. Dead or salvaged birds can be sampled for tissue by cutting off the end of the tongue or any other available tissue. A dead bird can also be frozen and shipped whole back to our lab for sampling. Please call for specific shipping instructions. Blood and tissue must be stored in either blood or tissue buffer, respectively. Samples can be stored at room temperature.

All samples and questions can be addressed to:

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