Laboratory and Field Sampling Protocols

Water Sampling:

Placing of the bottles on the wire:

1. Determine what the height of the sheave is from the water.
2. Lower the weight to the surface and zero the sheave.
3. First bottle placement is at 50m so take the height of the boat – difference of depth and 50m . Run that wire place the bottle.
4. Next 4 bottles are 10 meters apart.
5. Last bottle is always 9 meters apart. This is so the CTD is just under the surface.

Ex) For 62 m depth at Bouy B:

- Depth of 62m- 50= 12m
- 12m- 2m height= 10m. Run this much wire, place the bottle.
- Run 10 more meters, place the bottle
- Continue until last bottle
- Want 1 meter under surface, so only run 9 meters, place the bottle

ALL BOTTLES NEED THE MESSENGER IN BETWEEN!

Nutrient Sampling:

Field collection method:

1. Have pre-labeled 8 oz opaque acid washed Nalgene bottles ready for a sample from each Niskin bottle. Rinse each bottle with sample water 3x cap-on, then fill.
2. Add ~1 ml (to end of the taper on a disposable pipette) of chloroform. Generous swirl, PUT ON ICE!

Filtering is best done in the lab because of space constraints on the fishing vessels.

Supplies needed from the Loder Lab for collection and filtering:

- 1 acid washed filter canister with one 0.45μm filter for each station
- 1 acid-washed opaque Nalgene bottle for each depth sample
- 1 small bottle with ~125 ml of chloroform to preserve each sample
- 1 acid-washed screw-cap 50ml vial for each filtered sample

Our Lab supplies:

- 2 disposable pipettes
- 1 Pump Mastaflex
- 1 ring stand
- 1 tub to catch water from filter (or use sink)
- Cooler of ice from Rudman Hall

Laboratory Filtering Method:

Best done at end of sample day!

3. The Pump Mastaflex should be set up already. If the pump isn't set up then run the special tubing around the round part and then clamped down carefully with the screw clamp on the top. The stiff end of the tubing goes in the sample bottle, and the other end connects to the filter canister. Use the ring stand to hold the filter end up high. Run some DDW through the system to clean out the "pipes".
    - Special trick to preserve the filter: always remove the filter before changing the medium, change the medium then allow the air to expel and new medium to come through before reattaching the filter. Forced air tends to waste the filters.
4. Peel of the label from the Nalgene sample bottle and restick onto the green screw-cap acid washed vials that Loder lab supplied. Rinse the tubing and vial with filtered sample 3x, fill the sample vial to just below the line.
5. Between each sample rinse with DDW, but can keep the same filter.
6. Finished samples go upstairs in the Loder lab chest freezer, just to the right of the door. Leave a voicemail and note that they are there.

Chlorophyll Analysis:

Field collection method:

1. Have a clean pre-labeled 250ml Brown Nalgene bottle ready for each depth.
2. Fill each respective bottle with a water sample from the Niskin bottle. PUT ON ICE
3. ~100 ml water sample goes into small clean labeled glass jar and Lugol's added until is a weak tea color.

- Lugol's solution recipe:
  - ✓ 20g potassium iodide
  - ✓ 10g iodine crystals
  - ✓ 180 ml distilled water
  - ✓ 20ml glacial acetic acid

Laboratory Filtering and Analysis: MUST DO IN THE DARK!
Filter immediately after the sample day, and then put in Bucklin's freezer overnight.

4. Set up the Gast pump to the manifold and the large carboy. Carboy has two nipples on top, one of which is for the tubing from the bottom of the manifold and the other goes to the right side of the pump. Must use thick walled tubing so it doesn't collapse.
5. With clean forceps, place a clean 25 mm glass fiber filter on the top of the manifold, make sure the cap is back on securely.
6. Pour 100ml exactly of sample into each respective manifold cup. Pump away.
7. Use clean forceps to fold each filter and place into its respective centrifuge tube and add 10ml of acetone. Crunch the filters to loosen up the material.
8. Place into a regular freezer for no more than 2 to 24 hours.

Analysis:

9. Let the samples warm to room temperature to reduce the fogging that will form on the cuvette (MUST BE KEPT DARK!)
10. Warm up the fluorometer for 30 minutes.
11. Centrifuge the samples next door, 8 at a time, at a speed of ~1,600 for about 5 minutes. Want to see all settled material.
12. Fill a cuvette carefully, wipe its sides clean and place into the fluorometer.
13. Hit the "*" button and read the results.
14. Acetone disposal is in the hood.

- Calibration of the fluorometer should occur every 6 months. A 90% acetone blank should be used to zero the machine.

Zooplankton Sampling and Analysis:

Remember there are two casts!

- Formaldehyde solution is buffered to saturation with either Borate, in grocery store cleaning section, or Sodium Tetraborate Decahydrate. Use the buffered formaldehyde solution, by pouring off the clear stuff from the top.

1. Field sampling is with a 0.75m, 200μm mesh ring net vertical tow. No CTD is necessary. Run wire through the measured sheave, preferably amidships and attach the net and weight. Make sure there is a tagline attached to the top of the net ring and the weight the enough slack.
2. Drop the net until there is slack in the wire then retrieve at an average rate of 40 m min^{-1}. The captain should maintain the sampler in the lee and with a maximum way of 1 knot.
3. Once on board, rinse the net down with gentle salt water, probably the ships deck hose, then disconnect the cod-end, swirl gently then pour sample into the 32oz sample jar. Buffered formalin concentration is 40% so for 32 oz jars the formalin should be 40ml; for 16 oz jars the formalin should be 20ml. Bring a large volume squirt bottle so that the cod-end can be rinsed with minimal saltwater for removing the sample.
    - Gelatinous macro-zooplankton should be quantified then removed if necessary, a sub-sample (8oz jar) preserved for identification and proportion. Use a large beaker to measure the amount removed, record the subsample.

Laboratory Analysis:

- Pour the sample into a sorting tray and remove all gelatinous organisms larger than 1cm. Get volume.
  DRY WEIGHT:

1. Pour formalin sample into the Folsom splitter under the hood. Use a white plastic stirrer and agitate the sample to get everything distributed. Slosh the sample gently 12-13 times, then carefully dump.
2. ½ of the sample goes to dry weight the other ½ goes into an 8 oz jar for zooplankton taxonomic analysis.
3. Test the calibration of the micro-scale with the pre-measured Petri dish.
4. Label a Petri and weigh with two filters (of .202mm) inside. Record that starting weight.
5. Place a filter under the filter cup and screw back on.
6. Pour the ½ aliquot into the filter cup. Rinse the sample with 100ml of tap water and squish the sample around with the white spatula so it drains.
7. Pull that filter and all its contents and put into the pre-labeled and weighed Petri dish.
8. Place the second weighed filter under the filter cup and screw that back on.
9. Rinse out the remainder of the sample with the water squirt bottle into the filter cup. Also now rinse the tools used.

10. Place this second filter into the Petri, put the cap underneath and place into the oven at 60^{o} C for 48 hours.
11. Once done baking weigh the sample to the nearest 1000^{th}.
12. Throw out when done.

Taxonomic Analysis:

- The target subsample N is 200 overall organism count
- Additional aliquots for 75-100 Calanus spp or 150-200 Calanus spp if several stages are present.
- All Calanus spp should be staged and all others identified to species whenever possible.

1. Once are sure the dry weight analysis has worked, and then combine the two halves left over from the initial split into one sample.
2. Drain the sample, and then rinse with fresh water.
3. Dilute the sample with fresh water to a reasonable dilution that one draw with the Stemple pipette will not yield too high a number of animals. Record that total volume.
4. Use only the 5 ml volume aliquot thimble, stir with the pipette then sample.
5. Empty out the aliquot into a small container, transfer to the counting tray.
6. Identify all copepods, sex all mature copepods.
7. For the Calanus spp., stage and sex all.
8. Repeat, recording the counts PER ALIQUOT until the target totals are reached.

- All other zooplankton are to be classified as follows:
  - Amphipods (genus)
  - Bivalves
  - Chaetognaths (genus)
  - Coelenterates (genus)
  - Ctenophores (genus)
  - Cladocerans(genus)
  - Decapods (adults: genus; larvae: group)
  - Echinoderms (larva or juvenile)
  - Euphausiids (species)
  - Fish eggs (stage and species)
  - Fish larvae (species and size)
  - Larvaceans (genus)
  - Mysids
  - Ostracods
  - Polychaetes
  - Pteropods