**University of Washington, Marine Chemistry Laboratory**

Please read and familiarize yourself with your procedure before sampling. If you have any questions or need any clarifications on your sampling protocol, please don’t hesitate to contact us beforehand. (206)543-9235.

Happy sampling!

Kathy, Aaron, and Loren.

**Sampling Procedures for:**

* [**Chlorophyll A**](#Chlorophyll)
* [**DOC**](#DOC)
* [**POC/PN**](#POC_PN)
* [**TSS**](#TSS)
* [**Nutrients**](#Nutrients)
* [**TNP**](#TNP)
* [**Dissolved Oxygen**](#Oxygen)
* [**Dosimat Operation**](#Dosimat)

**Sampling Procedure:**

**Chlorophyll a**

Equipment:

Clean bottles

Filtration apparatus

Graduated Cylinder(s)

GF/F filters

Procedure:

Take water sample directly into a clean (acid-washed, rinsed 3X with distilled water) bottle. Pre-rinse bottle 3X with sample water prior to sampling.

Water samples should be stored in the dark and chilled until filtered.

**For best results, filter the water as soon as possible**, ideally right away, but THEY SHOULD BE FILTERED W/IN 24hrs!!! MAX!!!

Vigorously shake bottle then filter a **known volume** of water through the GF/F filter-and **record the filtration volume!**

Need to filter enough so that there is a small amount of color on the filer. As with any analysis that requires filtration, the more you can filter, the better, but you also don't want to clog the filter.

Fold filter in half and store in the supplied 15ml screw top tubes. Store/ship frozen and minimize the filter’s exposure to light.

Paper log sheets must accompany samples received in the lab.

Log sheet information must include:

Billing/Contact information, affiliation, sample ID, site and date, **filtration volumes**, and whether the sample is from a marine (oceanic, coastal, Puget Sound, estuary etc.) or freshwater environment (lake, stream pond etc.)

**Sampling Procedure:**

**Dissolved Organic Carbon (DOC) Samples.**

Equipment:

40ml glass “EPA” vials

Filtration apparatus

25mm carbon cleaned GF/F filters (combusted @ 450C for 4hrs.)

Procedure:

Take water sample directly into filtration apparatus

Filter **20-30mls** (about ½ to 3/4 full) through the carbon-cleaned filter directly into the DOC vial. It’s CRITICAL that the volume fall within this range. A good “eyeball” estimate would be to fill the vial to **JUST BELOW THE TOP OF THE BAR-CODE STICKER.** DO NOT completely fill the vial!!!

Place in freezer and ship frozen.

**Regarding freezer storage: We occasionally have had problems with sample vials cracking during the freezing process. If this occurs, the integrity of the sample will be compromised. In order to help prevent vials from cracking, we suggest storing the vials in a fridge to pre-chill the sample and vial, thoroughly wipe off any moisture and/or condensation on the outside of the vial just prior to placement in a freezer and freeze the vials upright or at a slight angle. If you’re really concerned about it, you can simply cap the vial tightly and store them upright in a fridge; however there may be biological effects to the OC if they are stored in this manner.**

If aqueous TOTAL organic carbon (TOC) samples are desired-DO NOT filter at all. HOWEVER, if there are a significant amount of particulates in the sample, you’ll have to filter the sample and keep the filter for PARTICULATE C analysis.

HOMOGENEITY of the sample cannot be guaranteed if there are particulates in the vial---SETTLING **WILL** OCCUR!

**PAPER log sheets are required upon submission of samples.**

Log sheet information must include:Billing/Contact information, affiliation, sample ID/Date, AND whether sample(s) are freshwater or saltwater, ideally an approximate salinity should be given.

**Sampling Procedure:**

**Particulate organic carbon/particulate nitrogen (POC/PN) Samples.**

Equipment:

Carbon Cleaned (baked @ 450C for 4.5hrs.) 25mm GF/F filters

Filter rack or 60ml syringe w/filter holder

Filter forceps

Analyslides or foil

Graduated cylinder

250ml sample bottles

Procedure:

Rinse sample bottle 3X with sample

Fill sample bottle almost full

Shake the sample to distribute particulates

Rinse graduated cylinder with some of the sample then measure 100-200mls of sample (this depends on the amount of particulate matter/biomass in the water; for PN the more filtered the better the signal, but you do not want to clog the filter either.

Filter a known volume of sample thru the C-cleaned filter.

Rinse filter cup w/DIW to make sure all of the particulate matter is rinsed onto the filter

Fold filter in half (filtered side in) and place in an analyslide or foil packet.

Label and freeze the sample for analysis

It is generally good practice to do up to 3 procedural blanks (ideally 3). Filter a similar volume of DIW thru the C-Clean filters and store as above.

Paper log sheets must accompany samples received in the lab.

Log sheet information must include:

Billing/Contact information, affiliation, sample ID/Date, **filtration volumes**.

**Sampling Procedure:**

**Total Suspended Solid (TSS) Samples.**

Equipment:

Filtration apparatus

Graduated Cylinder(s)

Pre-weighed GF/F filters

Procedure:

Filter a known volume of water through the pre-weighed GF/F filter.

Need to filter enough so that there is a good amount of stuff on the filter. As with any analysis that requires filtration, the more you can filter, the better, but you also don't want to clog the filter.

Fold filter in half and store in its OWN numbered analyslide. DO NOT mix-n-match filters w/other numbered analyslides!!

OK to store/ship at room temperature or chilled.

Paper log sheets must accompany samples received in the lab.

Log sheet information must include:

Billing/Contact information, affiliation, sample ID/Date, **filtration volumes**.

**Sampling Procedure:**

**Salinity Samples.**

Equipment:

250ml narrow-mouth glass bottles

Salinity is one of the most accurate analyses, measured to three decimal places. Salinity samples are used to calibrate the CTD, so proper sampling technique is essential.

Salinity samples should be taken after the gas and nutrient samples are taken from the rosette bottles.

Ideally, 250ml plastic coated narrow mouth glass bottles with leak proof caps should be used. Other bottle types (plastic) may be used but the data quality may be compromised and we need a **minimum volume of 125mls**.

Rinse the sample bottle 3X with sample.

Fill the bottle 80-90% full.

DO NOT FILL TO THE TOP!!! AIR SPACE MUST BE LEFT FOR PROPER ANALYSIS ON THE SALINOMETER.

Cap tightly and store upright in the case.

Paper log sheets must accompany samples received in the lab.

Log sheet information must include: Billing/Contact information, affiliation, sample ID/Date.

When using the UW salinity bottles and cases:

Please place salinity samples upright in the case. The inverted bottles indicate that the sample has already been run and are ready for the next sampling.

**Sampling Procedure:**

**Nutrient Samples.**

Equipment:

Sample bottles (60 ml HDPE)

60 ml syringes

Syringe filters (surfactant free cellulose, 25mm, .45micron pore size, Nalgene)

***PROCEDURE***

From the rosette bottle, draw water into the sample bottle to rinse the bottle twice...don't forget to rinse the cap too

Remove the plunger from the syringe and rinse the syringe with sample water twice

Fill the syringe fully with sample water...insert plunger

Invert syringe and expel the air bubble

Attach a filter to the syringe; filter about 5 ml of sample into sample bottle to rinse

Filter about 45-50 ml of sample into the nutrient bottle...the bottle should be NO MORE than 2/3 full

DO NOT OVERFILL THE BOTTLE!! WATER EXPANDS WHEN FROZEN AND IF THE BOTTLE IS TOO FULL THE ICE WILL FORCE ITS WAY OUT OF THE CAP AND TAKE THE NUTRIENT S WITH IT.

Securely cap the bottle and freeze upright in a -10 degree freezer.

Discard filter

Make sure you have filled out log sheets legibly and included them with the samples when you bring them to the lab for analysis.

Log sheet information must include: Billing/Contact information, affiliation, sample ID/Date, AND whether sample(s) are freshwater or saltwater, ideally an approximate salinity should be given.

**Sampling Procedure:**

**Total Nitrogen/Phosphorous (TNP) Samples.**

Equipment:

20ml Graduated Cylinder (The TNP Sampler)

Wide-Mouth Polypropylene (PP) 60ml bottles

Sharpie Pen or Marks a lot Magic marker

Procedure:

Take water sample directly into TNP sampler (pre-rinse 2-3X w/sample)

Pour 20mls into the bottle. If you’re sampling from a freshwater or high organic site, take 40mls.

Label bottle with marker or record the bottle #--DO NOT label with tape or paper. Bottles will be autoclaved and tape and other labels cannot withstand the temperature and pressure.

Paper log sheets must accompany samples received in the lab.

Log sheet information must include:

Billing/Contact information, affiliation, sample ID/Date, AND whether sample(s) are freshwater or saltwater, ideally an approximate salinity should be given.

**Oxygen Sample Collection and Treatment**

Samples for the determination of dissolved oxygen should be taken from the water sampling bottle immediately upon its arrival on deck. The sample should be collected in a calibrated, borosilicate glass flask with a ground glass stopper. Two types may be used—bottles approximating 250ml and bottles approximating 125ml. In the case of the latter, the entire contents of the flask is eventually used for titration while with the former, an aliquot is taken. We generally use 125ml (approx.) clear glass bottles that are calibrated to contain with an accuracy of +/-0.04ml and titrate the entire bottle as suggested by Carpenter (1965). Tubing used for oxygen sampling should be pre-soaked in seawater before using to minimize bubble formation in the sampling tube.

To fill the oxygen bottle from the oceanographic sampling bottle (e.g., a Niskin bottle), you will need a length of amber surgical tubing (or similar material) with an inside diameter of approximately ¼”. The length of this tubing should be approximately 12”, but keep the length as short as you can without sacrificing ease of movement while filling the oxygen bottle. To one end of this tubing attach a length of stiff plastic tubing or glass tubing that is a bit longer than oxygen bottle. You are now ready to begin the challenging task of drawing a high quality oxygen sample without contamination while bathed in an atmosphere that is about 21% oxygen!! Believe it or not, it can be done, and with a bit of practice ***you*** can do it!

A method that I have used will be described next, but remember the principle, which is to minimize contact of the seawater sample with the atmosphere. With this basic principle in mind, you can modify my technique to fit your particular blend of motor skills. When I draw an oxygen sample, I proceed as follows:

1. Attach the soft tubing to the spigot of the Niskin (or other type of bottle), and open the air vent on the Niskin bottle.
2. Open the spigot valve and flush the sampling tubing so that all air bubbles are removed. I find that the easiest way to do this is to hold the tubing in a straight line (more or less) and point the tubing downward while letting the water flow through the tubing at maximum velocity for a brief period. At this point, you control the velocity of flow by pinching the soft tubing with your fingers. If the tubing is not completely free of bubbles after the first brief period of maximum velocity, repeat the process one or more times by turning the flow on and off by pinching and unpinching the tubing. Some prefer to try to remove the bubbles from the tubing by holding it in a U-shape with the open end up and letting the water flow gently through the tubing. Any method of combination of methods that removes all of the bubbles from the tubing is OK.
3. The next step is to rinse your oxygen bottle while minimizing contact with the atmosphere. I do it by allowing the water to flow at a moderate pace out of the bubble-free tubing, inserting the tubing so that the stiff portion touches the bottom of the bottle, and then inverting the bottle. While in the inverted position, I move the stiff part of the tubing around so that a moderate “sheet flow” rinses the entire bottle with minimal turbulence.
4. Next, I momentarily stop the flow by pinching the tubing and inverting the bottle quickly. Then I start a moderate flow again and let the bottle fill as quickly as possible without forming a lot of turbulence while keeping the stiff part of the tubing near the bottom of the bottle. As the bottle begins to overflow, I let the overflow water rinse the ground glass stopper. After about half a bottle volume has overflowed, I begin to withdraw the tubing from the bottle by steadily raising while allowing another bottle volume (approx.) to overflow and continue to rinse the stopper.
5. Now I immediately add 1mL of the MnCl2 by placing the tip of the delivery pipette just below the surface of the sample in the oxygen bottle. This step is immediately followed by the addition (in like manner) of the NaOH-NaI. Both reagents are much denser than seawater, and they sink to the bottom and displace the upper 2ml of seawater in the bottle, which is helpful since the upper water has been in contact with the atmosphere. It is best not to immerse more than about 1/8” of the tips of the delivery pipettes since this should help to cut down on contamination. Not immersing the tips at all increases the possibility of contamination from the atmosphere.
6. Now, place the stopper in the bottle without trapping bubbles. I have found that the easiest way is also the way that works the best. Just drop the stopper into the bottle from a height of about one inch! If you place the stopper in the bottle slowly and carefully, you are more likely to trap a bubble. The stopper displaces the upper few mls of liquid in the neck of the bottle, thereby removing most of the atmospheric contamination that may have accumulated.
7. Now push down on the stopper to make sure that it is tight, and closely inspect the bottle to make sure that it is free of bubbles. A bubble the size of a pencil eraser will completely invalidate any sample, and much smaller bubbles can also totally ruin a sample depending on ambient concentrations and the accuracy that you desire. Any bubbles = bad sample, period.
8. Keeping a finger over the stopper, invert the sample several times to mix the first two reagents with the sample. A precipitate will form. Put the flask back in the bottle case, fill the flange with DIW, and allow the sample to settle for at least 15 minutes. Repeat. Samples should be kept cool and dark to prevent undesired photochemical reactions. DIW is added to the flange to act as a barrier to atmospheric oxygen. Stored in this manner, the samples are stable for 24 hours.
9. Add 1ml H2SO4. This can be done right before titration if within 24 hours of sampling or 20 minutes after the second shaking (step 8) if longer storage is needed (>24hrs.). If the precipitate is not completely dissolved when you are about to start your titration, just add another drop of acid.

Oxygen sampling NOTES:

1. Occasionally after the addition of sulfuric acid, a gas bubble will appear. This bubble is composed largely of carbon dioxide and a little nitrogen which may have been liberated from the solution. The former results from the chemical reaction of the acid and the carbonates. The presence of the nitrogen is accounted for by the reduced solubility of this gas upon the addition in electrolytes of the reagents and the possible increased temperature of the sample.
2. In running determinations of dissolved oxygen on fresh or slightly brackish water, considerable difficulty may be experienced in obtaining complete solution of precipitate. However, solution may be readily affected by the addition of a few crystals of sodium chloride to the acid solution.
3. In limnological studies, the use of hydrochloric acid may be substituted for the sulfuric acid. (Concentrated HCL is 12N and concentrated H2SO4 is 36N).

Procedure for

Oxygen Titrations

Please note—

This is analytical, not cookbook chemistry! The glassware and equipment—standard sample bottles, pipettes, stir bars, and buret tip—must be kept **SCRUPULOUSLY CLEAN**.

The glassware must be rinsed with hot water after use. **DO NOT LEAVE IT FOR SOMEONE ELSE TO DO**.

Sloppy analytical technique = nonreproducible numbers = bad grade in the class.

Dosimat Instructions

To turn on dosimat:

1. Press **FILL** button at the same time you turn on **POWER** button (red button in back).

2. Press **GO**.

3. Press **CLEAR**.

— Display should read **DOS 0.000 ml**.

To prepare to titrate:

1. Gently lift amber bottle of thiosulfate—shake—then replace in dosimat.

2. Turn dispense speed knob to 10. Dispense 15 ml thiosulfate to flush out the buret (3-5 ml aliquots) by pressing hand control button.

3. Turn dispense speed knob to 1.

4. Press **CLEAR** button.

5. Rinse off buret tip with DIW.

6. Make sure there are no bubbles in buret or moving bubbles in line leading to buret tip.

7. Turn on stirrer to 4.

Standard Preparation

1. Fill clean standard sample bottle 3/4 full of distilled water.

2. Add 1 ml H2SO4, mix well.

3. Slowly add 1 ml NaOh-NaI, mix well.

— If sample is not clear, discard and start again.

4. Using 10 mlA volumetric pipette, add 10 ml KIO3 standard.

— Always shake the standard before pipetting.

— Pour ~20 ml into small plastic beaker.

— Draw standard from the small beaker.

— Remove the pipette from the beaker. **NEVER DRAIN LIQUID BACK INTO STANDARD BOTTLE OR BEAKER**.

— Wipe down tip of pipette with kimwipe.

— Dispense into sample bottle. Do not put tip of pipette against wall of sample bottle—this will cause excess standard to be delivered from pipette.

5. Position sample bottle on stirrer; make sure buret tip is under the surface of the sample.

6. Make sure dosimat reads **0.000 ml** (press **CLEAR** to zero).

7. Titrate sample by dispensing thiosulfate into the sample.

— Use the thumb-button gizmo to dispense thiosulfate.

8. When the sample is light yellow in color, add 1 ml starch indicator.

9. Titrate to endpoint.

— Endpoint is when all color is gone; watch vortex in upper half of bottle.

— The endpoint is subtle—the difference between clear and sparkling clear.

10. Record endpoint.

11. Remove sample bottle; dispense a few drops of thiosulfate through buret tip to flush out any sample residue.

12. Rinse down buret tip with DIW.

13. Press **CLEAR** to zero dosimat.

14. Run at least 3 standards; at least 2 out of 3 should agree ± .001 ml.

15. After analysis, rinse bottles with hot water and store filled with distilled water.

Blank

The blank is a correction factor. The reagents may add a contamination to the standard and sample measurements. This contamination may be due to impurities in the crystalline form of the reagent or because the liquid reagent was contaminated by “something” (seawater, sunlight, another chemical) while in use aboard ship.

To prepare a blank:

1. Fill a standard sample bottle 3/4 full of distilled water.

2. Add 1 ml H2SO4, mix well.

3. Slowly add 1 ml NaOH-NaI, mix well.

4. Add 1 ml MnCL2, mix well.

5. Using automatic pipette, add 1.0 ml KIO3 standard.

6. Add starch immediately (because the sample is light yellow).

7. Position sample bottle on stirrer; make sure buret tip is under the surface of the sample. (Titrate slowly; remember this is only 1/10 as strong as the standard.)

8. Make sure dosimat reads **0.000 ml** (press **CLEAR** to zero).

9. Titrate sample by dispensing thiosulfate into the sample.

— Use the thumb-button gizmo to dispense thiosulfate.

10. Titrate to endpoint.

— Endpoint is when all color is gone; watch vortex in upper half of bottle.

— The endpoint is subtle—the difference between clear and sparkling clear.

11. Record endpoint #1.

12. Press **CLEAR** button.

13. Add 1.0 ml more of KIO3 standard.

14. Titrate to the endpoint #2.

15. (Endpoint #1) – (Endpoint #2) = blank correction factor

Translation

Endpoint #1 (in ml) = volume of thiosulfate needed to titrate the first 1 ml KIO3 + reagents.

Endpoint #2 (in ml) = volume of thiosulfate needed to titrate the second 1 mlKIO3.

Therefore (Endpoint #1) – (Endpoint #2) = volume of thiosulfate needed to titrate reagents.

This value may be negative or positive or zero.

Titrating Samples

1. Carefully remove cap, rinse glass stopper.

2. Add clean stir bar.

3. If it has not already been done (if the acid has not been added, there will be a precipitate in the sample), add 1 ml H2SO4, mix well.

4. Position sample bottle on stirrer; make sure buret tip is under the surface of the sample.

5. Make sure dosimat reads **0.000 ml** (press **CLEAR** to zero).

6. Titrate sample by dispensing thiosulfate into the sample.

— Use the thumb button gizmo to dispense thiosulfate.

7. When the sample is light yellow in color, add 1 ml starch indicator.

8. Titrate to endpoint.

— Endpoint is when all color is gone; watch vortex in upper half of bottle.

— The endpoint is subtle—the difference between clear and sparkling clear.

9. Record endpoint.

10. Remove sample bottle; dispense a few drops of thiosulfate through buret tip to flush out any sample residue.

11. Rinse down buret tip with DIW.

12. Press **CLEAR** to zero dosimat.