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Dear colleague:

Please read through the following material on the proper collection methods for high-quality inorganic carbon analyses. The following pertains to the proper collection methods for dissolved inorganic carbon (DIC) and total alkalinity (TA) samples and does not address requirements for *p*H and pCO_2 sampling. The PMEL Carbon Program collaborates with a wide range of partners with different objectives associated with their individual research projects. Please keep your particular needs and goals in mind as you read through this document, as not all suggestions and remarks will apply equally to all projects. We have tried to make the relative benefits and requirements for each different application clear, but please ask us whatever remaining questions you may have upon reading this. We will use your feedback and questions to improve future iterations of this document and to ensure that you understand what is needed to properly collect your samples.

Best regards,
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INORGANIC CARBON SAMPLING: PLANNING AND SAMPLE COLLECTION

Section 1: Considerations in planning your field sampling

Bottle size—In order for us to run both dissolved inorganic carbon (DIC) and total alkalinity (TA) analyses on the same sample, we need a 500 mL sample. If we only need to run one of the two analyses (DIC *or* TA), a 250–300 mL sample will suffice. Most people elect to have both DIC and TA analyses run, so *please be sure to specify if you will only want one of the two analyses when submitting your sample request by selecting the 250 mL bottle size and indicating which analysis you need in the remarks box* (www.pmel.noaa.gov/co2/files/pmelcarbonsamplebottlerequestform.pdf)

Water sampler size—In order to collect your samples properly, you will need a water sampler that can collect several times the volume of water needed to completely fill the sample bottles, in addition to the volume needed for any other samples to be collected from the same bottle. This is because the collection method involves overflowing the sample bottle with two full exchanges of water to collect a clean sample. Also the sample must not include the last water to come out of the sampler, which will have been exposed to air as the sampler is emptied into the sample bottle and will thus be contaminated. To collect a 500 mL sample, the smallest acceptable water sampler would be a 2 L Niskin (vertical style bottle, below left) or Van Dorn sampler (horizontal bottle, below right) if you are exceptionally careful in collecting the sample, but a 2.5 L water sampler or larger would be preferable to ensure sample integrity. For the few people who may need the 250–300 mL sample bottles, the smallest acceptable sampler would be 1 L, but a 1.5 L sampler would be preferable (the volume is not as critical if you will only be collecting TA samples). *Note: these are the volumes required solely to collect the DIC and/or TA samples—to collect additional samples requires a larger water sampler and/or multiple casts.*

Items you need to provide—We cannot provide the following items: mercuric chloride for sample preservation, a pipette or repipettor for dispensing mercuric chloride solution, and a water sampler. Information about various appropriate pipettes and repipettors can be found on our web site (http://www.pmel.noaa.gov/co2/story/Laboratory+analysis+details). Remember to also order pipette tips of the appropriate size if you choose to use a pipette. If you will be collecting a lot of samples, it may be more convenient to use a repipettor. However, they typically come attached to rather large glass bottles, so they may not be appropriate for use if you only need to collect a few samples at a time, as you would need to prepare and subsequently dispose of a larger volume of mercuric chloride solution if you do not use it all. *Note: Mercuric chloride solutions must be handled as hazardous materials and sent to an EPA-approved facility.* Repipettors may also be unsafe for use in some small boats or anywhere they cannot be secured to prevent breakage.

Items we will provide—We will supply the sample bottles (250–300 or 500 mL size) in cases padded for their safe transport (*please indicate whether you will be hand-carrying or shipping them in your sample request*, *as we have both large, well-padded cases for shipping and more compact, less well-padded cases for hand transport*); a sampling tube (or "noodle"); grease, clips, and rubber bands for sealing the sample bottles; and a paint pen for labeling the bottles.

Transporting samples containing mercuric chloride ($HgCl_2$) as a preservative—Inorganic carbon samples must be preserved using mercuric chloride, which is a toxic chemical and thus requires special handling. For information on shipping samples with a very low concentration of $HgCl_2$ in them, please see the links on our web site to information about shipping and small quantity exceptions (http://www.pmel.noaa.gov/co2/story/Laboratory+analysis+details).

Information we require to process your samples and perform desired post-processing analysis—In order for us to analyze your samples, we require certain ancillary measurements that depend on the objective of your measurements:

- If you are 1) doing manipulative experiments to study organismal response to ocean acidification conditions in a lab or field setting or 2) researching or monitoring ocean acidification conditions in the field, we can provide a ***full characterization of the***

inorganic carbon chemistry of your samples. From the DIC and TA measurements, we can calculate all remaining inorganic system parameters—*p*H, pCO_2, fCO_2, aragonite and calcite saturation states (Ω_{arag}, Ω_{calc}), and concentrations of bicarbonate ion ($[HCO_3^-]$), carbonate ion ($[CO_3^{2-}]$), and dissolved carbon dioxide ($[CO_2]$)—using a program called CO2SYS. To do so, we require:

- *Water temperature (T)* at the time of sample collection.
- *Pressure (P) or depth* that the sample was collected from (i.e. the depth of the experimental tank, if the water collection spigot is located at the bottom, or the depth at which the CTD fired the Niskin bottle).
- *Salinity (S)*—We routinely measure salinity of all DIC samples using thermosalinographs integrated into our analytical systems, but if you have high-quality salinity measurements (i.e. from bottle analyses [best quality] or CTD measurements), please provide your salinity data to us when you return the full sample bottles to us or indicate clearly that samples should not be run until you give us this information. *Please note that we cannot run alkalinity samples until we have the final salinity values.*
- *Nutrients*—To make the highest quality calculations, we require concentration data for phosphate ($[PO_4^{3-}]$) and silicate ($[SiO_4^{4-}]$). Not having nutrient data can introduce an error on the order of 1–5% or possibly higher, depending on nutrient concentration, in the calculated pCO_2 values.

- If you are working with us to ***develop predictive models for describing ocean acidification conditions*** on the basis of proxy variables, we will need all of the above (i.e. T, S, P, $[PO_4^{3-}]$, and $[SiO_4^{4-}]$), in addition to a few other parameters:
  - *Oxygen concentrations*—On the West Coast, oxygen concentrations are among the strongest predictors for inorganic carbon variables under well-oxygenated conditions. High-quality oxygen measurements are required for this work and can consist of either Winkler oxygen data or CTD-O_2 data calibrated against Winkler O_2 measurements.
  - *Nitrate*—In upwelling systems or enclosed systems such as bays, nitrate $[NO_3^-]$ may play a critical role in limiting inorganic carbon fluxes and may thus be an important predictor variable, particularly where terrestrial inputs of NO_3^- are important.
  - *Other*—If there are other variables that you think may exert important influence on inorganic carbon cycling in your study locale, it will be important to quantify those factors so that it can be tested in the predictive models.

Section 2: Preparation prior to drawing your samples

- **Prepare a saturated solution of mercuric chloride ($HgCl_2$) in advance—**Read the MSDS for mercuric chloride. The saturation of mercuric chloride is 7.4 grams per 100 mL of water—*this is the minimum ratio you can use*. We suggest a 1:10 ratio for a saturated solution; e.g. 10 grams mercuric chloride per 100 mL water should be sufficient for 200 samples. Ensure that your pipette (or preferably your repipettor) is capable of dispensing 200 µL properly. Make sure that the solution stays saturated at all times by checking to see that crystals remain in the bottom of the bottle. If taking DIC and alkalinity samples separately, both samples must be poisoned with $HgCl_2$.

- **Label the sample bottles in at least two locations—**Bottle numbers can rub off, which results in loss of data for any samples that cannot be matched to your sample log. Each bottle should be labeled with a unique number (e.g. A1 to A20, PS-1 to PS-20, etc.) and be placed back in the storage crates sequentially. Please use bottle numbers of six characters or less. We strongly recommend using the DecoColor paint marker we have provided for labeling the sample bottles, as labels written with Sharpies (and similar) do not reliably remain clear and are easily rubbed off. ***Do not apply adhesive paper to the bottles!*** Please also indicate—either in the sample log sheet you send us or a separate list—which crate each sample is in. It will help you and us to keep track of where samples are. All of our crates are labeled (e.g. C43).

- **Grease the stoppers—**Apply a thin strip of Apiezon L grease around the bottom of each bottle's stopper (see picture at right for proper greasing method). After sampling, you will insert a stopper into the neck of the sample bottle and twist to spread the grease evenly and form a good seal. *Please be aware that this grease can contaminate samples for dissolved organic carbon analyses, so care should be taken to prevent this grease from getting onto shared water sampling equipment.*

- **Soak your noodles—**Soaking sampling tubing ("noodles") in a bucket of clean water before the first sample and between subsequent sample helps to prevent bubbles from forming in the noodle during sampling. *Remember: bubbles are the enemy of high-quality dissolved gas samples!* Noodles may be either a single piece of tubing or consist of a narrower piece of flexible tubing inserted into a larger diameter piece of rigid tubing, and held in place with a cable tie. For single-piece noodles, we recommend marking the noodle so that the same end is placed on the water sampler each time. For two-piece noodles, the rigid tubing goes into the sample bottle and the smaller, more flexible end slips onto the water sampler hose-barb or nipple.

- **Prepare your log sheets**—Prepare log sheets with all necessary information about locations and depths to be sampled, geographic coordinates, bottle numbers, etc. Remember to take into account the information that we require to process your

samples, which depends on your objectives (as described in Section 1):

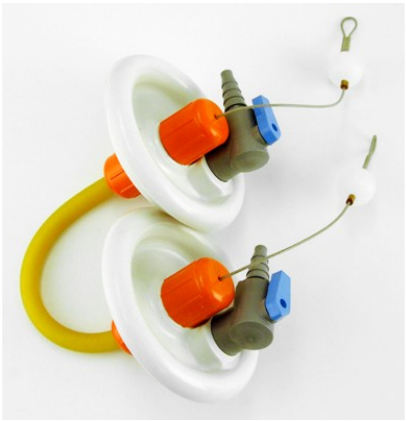
- *Full inorganic carbon chemistry only:* We require salinity, temperature, pressure or depth, and phosphate and silicate concentrations to make the necessary calculations.
- *Developing predictive models:* We require all of the above, plus oxygen and nitrate concentrations, and anything else that you think might be an important control variable.

An example of our DIC log sheet for major cruises can be found at:
http://www.pmel.noaa.gov/co2/story/Laboratory+analysis+details

Section 3: Sample drawing procedure

- **Draw samples immediately after bottles come aboard—**Dissolved gases must be sampled before other, less sensitive samples such as nutrients and salinity are collected. The correct order of sampling is oxygen first, then dissolved inorganic carbon parameters (pCO_2, pH, dissolved inorganic carbon, and alkalinity).

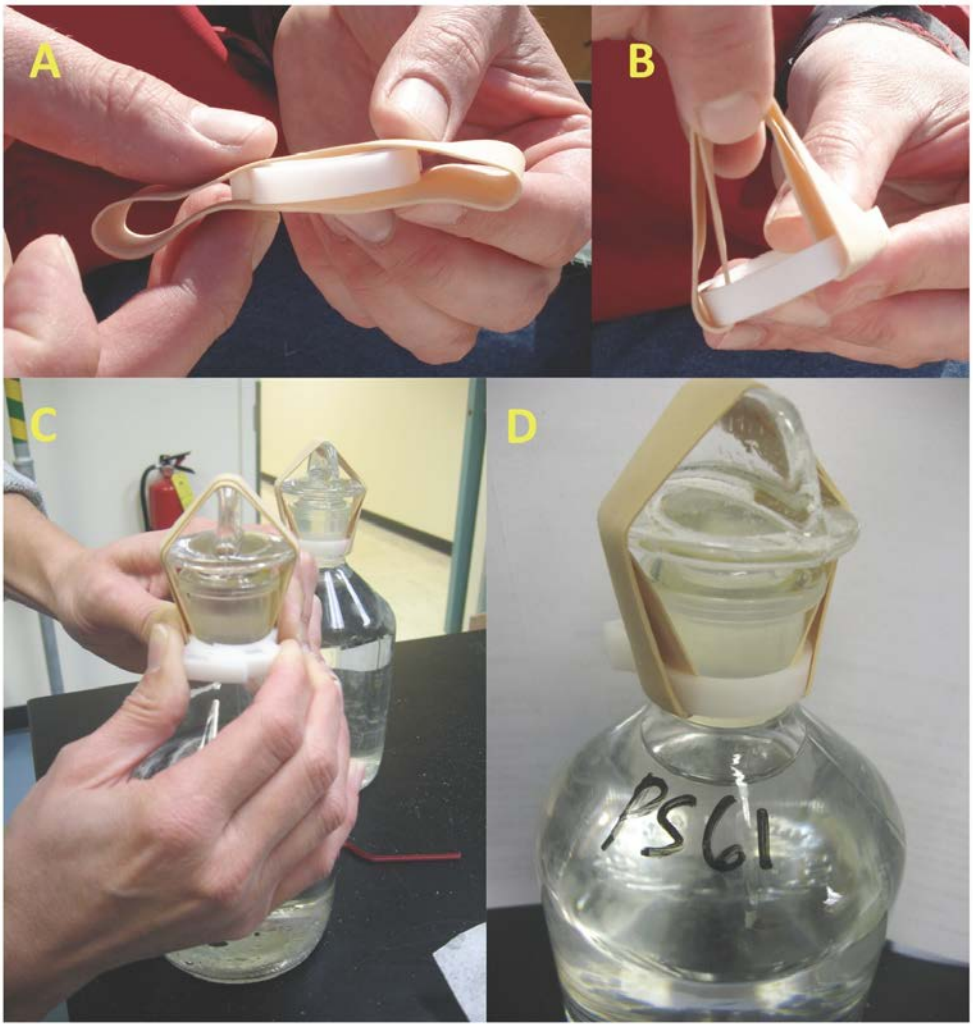
- **Check the water sampler for leaks—**Before opening the air valve on a bottle, open the sample valve by turning the stopcock switch (blue part on Van Dorn pictured below right) or pushing the outer petcock ring in (as on Niskins at left below). *If water begins to flow out or if water is dripping steadily around the end caps, the end caps did not seat correctly when the sampler closed, and the sample is compromised*. Check that the tubing between the end caps is in good condition and attached correctly. If no leaks are observed, close the petcock/stopcock after checking for leaks.



- **Fill sample bottle—**Attach the designated end of the Tygon tubing to the stopcock/petcock of the water sampler. Insert Tygon tubing to the bottom of the sample bottle, open air valve, open water valve, and begin water flow. Invert the bottle over the tube to rinse the bottle carefully with the sample water, moving

the tubing to eliminate any air bubbles on the bottle walls. *It is critical to prevent exposure of the sample to air bubbles.* Slowly right the bottle and begin to fill, pinching the tubing (if necessary) to control the influx of bubbles. Allow the bottle to fill completely and to overflow at least one full volume. Bend and pinch the tubing to stop the water flow while the tubing is still touching the bottom of the sample bottle. Then withdraw while the tubing is still bent and pinched—this creates a 'calibrated' headspace of ~1% to allow for sample expansion. Check the bottle for any bubbles; if you see any, discard the sample and redraw.

- **Carefully add 200 µL of $HgCl_2$** with pipette or repipettor to 500 mL sample bottles (100 µL for 250–300 mL bottles)—do not submerge pipette tip in sample.
- **Insert greased stopper into neck of bottle and twist** to form a good seal. *There should not be any streaks visible in this greased seal.*
- **Seal bottle with rubber band and collar** as follows (also see pictures below). *This is critical for proper storage.*
    - Place the whole collar through the middle of the rubber band (panel A).
    - Pull both sides of the rubber band through the middle of the collar (panel B).
    - Then, while holding the collar, pull the rubber band down over the stopper and pinch the collar tightly around the neck of the bottle (panel C). Be sure to pull the collar down so that it is below the neck of the bottle. You may need to use channel locks to close the collar tightly and secure the stopper and rubber band in place.
- **Invert the sample several times to mix the mercuric chloride thoroughly.**
- **Dip each bottle in a bucket of clean fresh water** up to the neck, dry, and place in storage crate. Bottles can be stored at room temperature but should be kept out of direct sunlight or high temperature. If cold storage is available, that is preferable, but samples should never be frozen.
- The sample in panel D has the correct volume of headspace and a properly fit clip and rubber band.
- Take duplicate samples as you see fit, e.g. 10% of your samples. *Consider taking duplicates of all critical samples.*


A
B
C
D
PSG1

Section 4: Returning the samples to PMEL

- Prepare samples for shipment or hand delivery to PMEL by packing any unused sampling supplies that are not needed for ongoing sampling in the cases with the sample bottles.
- Prepare a spreadsheet with all data needed by PMEL to process samples (see template on the web site— http://www.pmel.noaa.gov/co2/story/Laboratory+analysis+details).
- Email oar.pmel.co2.samples@noaa.gov to alert us that we should expect a delivery of samples from you, the expected arrival date, and by what means they will arrive (e.g. UPS or your car). We will respond with information about who to list as the recipient if you are shipping samples, as frequently one of us will be in the field. If you will deliver the samples, we need to have you on the security list, and you will need to provide photo identification at the security gate.
- Ship or deliver samples to:
    - <Name of recipient>
    - NOAA Pacific Marine Environmental Laboratory
    - 7600 Sand Point Way NE, Bldg. 3
    - Seattle, WA 98115
    - Tel: +1.206.526.6890
- Email your sample log/ancillary data spreadsheet to us, along with any tracking numbers at oar.pmel.co2.samples@noaa.gov.