“Standard Operating Procedures (SOPs)” for Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems (Draft)

SOP for Monitoring Seawater Carbonate Chemistry

SOP for Dissolved Inorganic Carbon (DIC) and Total Alkalinity (TA) Sampling

(This document has been summarized from other existing SOPs*.)

I. Overview of Procedure

In order to calculate all the parameters of the carbonate system (pCO₂, pH, carbonate species, saturation states (Ω), etc), both DIC and TA must be measured. As dissolved gas is to be analyzed, samples for DIC/pH/TA should be among the first collected. Samples are kept in borosilicate glass bottles and sealed well. Samples are poisoned with a small volume of saturated mercuric chloride (HgCl₂) solution.

Keep in mind that the most accurate carbonate system calculations require the following supporting information: salinity, temperature, pressure or depth, and phosphate and silicate concentration. Hence these information should accompany the samples.

II. Materials

The following materials are needed for DIC/TA sampling:

1. **Borosilicate glass bottles** - 500ml to run both DIC and TA, 250-300ml if only one parameter i.e. DIC or TA. Corning Pyrex reagent bottles with Pyrex stoppers; or Duran, Kimax, Pyrex media bottles with plastic caps may be used. Each bottles must be labeled properly on the shoulder of the bottle. Make sure labels used will not come off when bottles get wet.

   When using Pyrex reagent bottles with Pyrex glass stoppers, apply 4-5 thin vertical strips of vacuum grease (can use Apiezon L–ultrahigh brand) around each stopper. After sampling, insert the greased stopper into the neck of the sample bottle, and twist to spread the grease evenly and form an airtight seal.

   When using bottles with plastic caps (Duran, Kimax, Pyrex), tighten the plastic cap a second time after an hour of filling the bottle to ensure no sample leak and airtight seal.

2. **Water sampler** - In order to collect seawater samples properly, a water sampler that can collect several times the volume of seawater needed (at least 3) to completely fill the sample bottles for DIC/TA, plus ample volume for the other parameters is needed. 5L Niskin bottles (General Oceanics) may be used.

   DIC and TA samples must not include the last water to come out of the sampler, which will have been exposed to air as sampler is emptied, because this will result to contaminated samples.
3. Saturated solution of mercuric chloride (HgCl₂) - The saturation of HgCl₂ is 7.4g per 100mL of deionized water, but 10g per 100mL is more commonly used. HgCl₂ is hazardous material and should be treated as such in the laboratory, during sampling and transport.

4. Sample tubing - Samples are drawn from the water sampler to the glass bottles through a tubing. Tygon tubing may be used. The sample tubing is soaked in clean water before the first sample and between subsequent samples to prevent bubbles from forming in the tubing during sampling. Mark the tubing so the same end is placed on the water sampler each time.

* Dickson Lab Sampling Guide for Stoppered Bottles; DIC-TA Sample Collection CRED

5. Log sheets - Prepare log sheets with all necessary information about location and depths sampled, geographic coordinates, bottle numbers, etc.

III. Sampling procedure

1. Draw samples immediately after Niskin bottles come aboard. The correct order of sampling is oxygen first, then carbonate parameters, then other parameters (nutrients, etc). But before sample collection, check the Niskin bottle for leaks, make sure sample water is not leaking from around the end caps, air valve or stopcock. If no leaks are observed, collect the seawater sample.

2. Fill the sample bottle. Attach the designated end of the Tygon tubing to the stopcock nipple of the Niskin bottle. After rinsing the Tygon tubing prior to sample collection, insert the tubing into the bottom of the sample bottle, open air valve at the top of the Niskin, open water value (stopcock), and begin water flow. Invert the sample bottle over the tubing to rinse the inside of the bottle carefully with the sample, eliminating any air bubbles which may have stuck onto the walls of the sample bottle.

   Slowly right the sample bottle and keeping the tubing at the bottom of the bottle, begin to fill. Allow the bottle to fill completely and to overflow 3 full volumes (count how long it takes to initially fill the bottle and multiply this by 3). When full, pinch the tubing to stop sample flow while the tubing is still touching the bottom of the bottle. Then withdraw the tubing while still pinching the tubing. The volume displaced by the tubing creates a headspace of ~1% of the total sample volume, this allows for possible sample expansion during transport to the laboratory. Check bottle walls for any air bubbles. If there are bubbles, discard the sample and redraw from the Niskin bottle.

3. Using a pipette, inject 100µL of HgCl₂ into 250-300mL bottles (200µL for 500mL bottles). Do not submerge pipette tip into the sample or try to retrieve pipette tip accidentally ejected into the sample bottle.

4. If Pyrex bottle with glass stopper is used, insert the greased stopper into neck of the bottle and twist to form a good airtight seal between the stopper and bottle. Seal the sample bottle with rubber band and collar (if band is lost, use electrical tape over the stopper and under the bottle). Place the plastic 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). 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. This sample (panel D) has the correct volume of headspace and a properly fit clip and rubber band.
If glass bottles with plastic caps are used, cap the bottle tightly and make sure to re-tighten the plastic cap a second time after an hour of filling the bottle. This is to ensure no sample leak and an airtight seal.

5. Invert the sample at least 5 times to mix the HgCl₂ thoroughly.

6. Store the bottles at room temperature out of direct sunlight or high temperature. If cool/air conditioned storage is available, that is preferable. Samples should never be frozen.

7. Take duplicate samples at critical sampling sites (e.g. 10% of the total number of samples).

Panels A-D
Instructions for DIC/pH/ALK Sampling Program(s)
Dissolved Inorganic Carbon (DIC), pH or Alkalinity (ALK)

Overview of procedures

As a dissolved gas is to be analyzed, samples for DIC/pH should be among the first collected.

Samples are to be collected in Pyrex reagent bottles and are sealed using a greased glass stopper secured with a rubber band and clip.

Samples are to be poisoned with a small volume of a saturated mercuric chloride (HgCl₂) solution.

If at any time during the cruise, you run into a problem and/or want to discuss aspects of the sampling procedure, please email:

David Cervantes at d1cervantes@ucsd.edu
Or
Guy Emanuele at gemanuele@ucsd.edu

Remember that time is of the essence when sampling for DIC/pH. Please be sure to carefully read these instructions BEFORE you start sampling, and be sure to move quickly between the steps described herein.

Thank you very much for assisting with this sample collection.
Instructions for DIC/pH/ALK Sampling Program(s)

Dissolved Inorganic Carbon (DIC), pH or Alkalinity (ALK)

Before drawing the first sample, the following items should be removed from the sampling kit and prepared for use:

1) The polyethylene foam block, which has holes for holding a single bottle, stopper and a smaller bottle for mercuric chloride. If possible, this block should be mounted in a secure position with screws.

2) The 20 mL syringe and its ~2 inch Tygon tube. This will be used to withdraw enough water from the sample to create a ~1% headspace. There is a short piece of tubing on the tip of the syringe. Push the two-inch tube onto this piece of tubing if it is not already attached.

3) The grease dispensing "gun" and 30 mL syringe of grease and delivery tip. Install the 30 mL syringe of grease onto the dispensing "gun". Remove the orange cap from the end of the syringe and replace it with the green delivery tip.

4) Yellow Clamp Tool. This will be used to assist in securing the band and clip on each bottle.

5) Green Swab. This will be used to remove water from the neck of the bottle after filling.

6) The Eppendorf pipette and delivery tip. Install the tip onto the pipette. The Eppendorf has been set to a volume of 120 micro liters (about 0.02% of the sample volume) and will be used to dispense the saturated mercuric chloride solution.

7) Saturated mercuric chloride (HgCl₂) solution. This will be the solution used to poison each sample.

8) The plastic bag containing the Tygon drawing tubes. There are two sizes of tubing. Determine which size will be needed to draw samples from the Niskin bottles.


10) Open the blue plastic box containing bottles and remove the first two layers of polystyrene foam. These must be returned to the box after all samples have been drawn.

11) The box of kimwipes. This will be used to remove grease from the neck of the bottle and general space clean up if needed.
Instructions for DIC/pH/ALK Sampling Program(s)

Dissolved Inorganic Carbon (DIC), pH or Alkalinity (ALK)

Photos of the sampling procedure have been added to pages 6 through 11 for a visual understanding of the instructions as they are referenced in the steps below.

Sample Drawing

1) Remove the first bottle from the blue box. The box has a tag in the corner from which the first bottle should be removed. You will notice the bottle has been marked to show an ~1% airspace (important).

2) Remove the greased stopper from the bottle and with a kimwipe, remove as much grease as possible from the stopper. Using the grease gun, apply four thin beads of grease to the entire length of the frosted portion of the stopper (Figure A). Notice that each stopper is paired with a specific bottle. Both the stopper and bottle have the same number. It is very important that this pairing stays together as each stopper has been lapped by hand to fit its bottle (in extremely rare circumstances, numbered pairings may not be provided).

3) Put the re-greased stopper into the polyethafoam hold. The greased portion of the stopper should be up.

4) Using a kimwipe, wipe the grease from the neck of the bottle.

5) Find the appropriate sized tubing for the Niskin in which you are sampling from. Since the bottles have been cleaned and dried, there is no need to rinse the bottle before filling. If you are the first sampler drawing from a Niskin, open the vent to allow for water flow. Run water out the drawing tube, pinching the tube to eliminate any air bubbles that may adhere to the sides of the tubing. With the tubing pinched between your fingers, insert the end of the tube to the bottom of the bottle. Start the flow slowly until the bottom of the tube is covered with water then increase the flow until the bottle is being filled as fast as the water comes out. Overflow the bottle at least 50%. This is easy to do this by counting the seconds it takes to fill the bottle, then restarting the count until the bottle has overflowed the appropriate volume. Once overflowed the appropriate amount, pinch off the tubing so that water slowly comes out of the Niskin. Slowly lower the bottle to remove the drawing tube, leaving the bottle full to the brim (Figure B).

Creating an ~1% air space

Place the filled bottle in the polyethafoam holder and using the 20 mL syringe with ~2 inch tubing attached, draw out 16 mL of your sample. Set the syringe aside and proceed quickly to the next step for poisoning (Figure C, D).
Instructions for DIC/pH/ALK Sampling Program(s)

Dissolved Inorganic Carbon (DIC), pH or Alkalinity (ALK)

Poisoning the sample (addition of Saturated Mercuric chloride, HgCl₂)

1) Depress the top of the pipette to the first stop position. Put the tip of the pipette into the small plastic bottle of saturated mercuric chloride solution. Let out the top slowly to fill the tip. Look at the tip to be sure the tip has filled with solution. If not, eject the solution back into the bottle and try again (Figure E).

   ** Do NOT put the tip into the sample**

2) With the tip of the pipette as close as possible to the surface of the water in the sample bottle, push down the top to the first stop, then depress further to the second stop to "blow out" the remaining solution in the tip. Set the pipette aside (Figure F).

   [Special note: Although these tips are reusable (if they do not touch the sample), they can wear out. If the tip does not fill correctly, replace the tip with a spare and proceed with the poisoning. Once the stopper has been replaced and secured, the clogged tip should be discarded.]

3) Using the green swab, wipe away any droplets of liquid that have adhered to the greased neck of the bottle. **This is extremely important**. If all the water is not removed, the integrity of the subsequent seal will be compromised.

Replace and secure the stopper

1) Remove the greased stopper from the polyethafoam block and push the stopper straight down into the bottle. Allow the grease to spread until there is no air space between the strips then twist the stopper to complete the seal (Figure G).

2) Secure the band in place using one of the white clips and stretch the band over the top of the stopper (Figure H, I, J, K). The yellow clamp tool can be used to secure the white clip.

3) Mix the sample by inversion at least five times.

4) Put the sample into the sample box.

Recording data on the data sheet

The columns on the data sheet should be completed with the information available. Be sure to record the number on the bottle label in the appropriate column. Additional labeling of the bottle is not necessary.
Instructions for DIC/pH/ALK Sampling Program(s)
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Some additional notes

Should the dispensing grease gun fail, the grease can be applied to the stopper using the 20 mL syringe filled with the Apiezon-L grease. You can use a green tip on this syringe or not.

Should the Eppendorf pipette fail, the mercuric chloride can be added to the sample bottle using one of the 4.5 mL plastic disposable pipettes. Put the end of the pipette into the bottle of mercuric chloride and fill it about half full. Add three drops of mercuric chloride to the sample bottle. If you have to use this plastic pipette, be sure to make a note of this on the data sheet. Before you actually use it to add drops to a sample, you should practice dispensing drops back into the mercuric chloride bottle. You will find that with just a little practice, adding the mercuric chloride a drop at a time is relatively easy.

A box of small kimwipes has been sent for general wiping before, during, and after sampling. For example, with use, the piece of tygon tubing used to collect the sample from the Niskin will accumulate some grease from the neck of the greased bottles. As needed, use these wipes to remove the grease. Note: it is much easier to wipe the grease off the tube when the grease is warm rather than cold.

You can also use a wipe to remove any residual liquid from the tubing on the end of the syringe that is used to remove water from a sample bottle to create the headspace. You don’t want to transfer any liquid or salt from one sample to the next by failing to wipe this clean after each use.

If you spill some of the mercuric chloride, first, put on a pair of rubber gloves. Blot up the spill with 1 or 2 of the kimwipes. Put the kimwipe(s) into the gallon bag labeled "mercuric chloride clean-up wipes". This bag will be removed from the ship at the end of the cruise. Use a sponge to wipe down the area where the mercuric chloride spilled. Rinse the sponge thoroughly with tap water. As diluted, this very low concentration of mercuric chloride can be discarded. When finished with this clean-up, be sure to wash your hands.
Figure A
Positioning sampling equipment and greasing a stopper before filling a bottle. It is encouraged to secure all equipment to the bench top as weather can be unpredictable.

Figure B
The end of the tubing is at the bottom of the bottle and the tubing is being pinched to remove any bubbles inside the tubing.
Figure C
Placing the filled bottle into the polyethafoam block. Notice that all sampling equipment is already prepared and within arms reach.

Figure D
Creating the 1% headspace with the 20 mL syringe.
Figure E
Filling the pipette with saturated mercuric chloride.

Figure F
Dispensing the saturated mercuric chloride into the sample. The pipette tip must not touch the sample so it can be reused again. (Ignore the headspace in Figure F as it is larger than 1%)
Figure G
A stopper with four strips of grease is pushed down into a now ready sample bottle. After the stopper is pushed completely down and the grease has spread to eliminate air space, the stopper is twisted to complete the seal. (Ignore the headspace in Figure G as it is larger than 1%)

Figure H
Clip and band.
Figure I
Clip is inserted inside the band.

Figure J
With the clip still inside the band and the band above the stopper, the clip is pushed down until around the neck of the bottle.
Figure K
Once the clip is at the neck of the bottle, it can be clipped shut. It is also acceptable to shut the clip around the neck first and then pull the band above the stopper as opposed to pushing down (not pictured).