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# NATIONAL CORAL REEF MONITORING PROGRAM

## Standard Operating Protocol

*for*

## Water Sampling (Pacific - Climate)

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**NOAA**  
**CORAL REEF**  
CONSERVATION PROGRAM

## Pacific NCRMP CTD and water sampling standard operation procedure updated 06/2023

If available hands on deck and conditions allow, conduct the CTD cast and the water sample collection simultaneously.

### MATERIALS NEEDED

- GPS unit
- CTD (RBR concerto3) unit and line (with depth markings)
- 5L Niskin Bottle and line (with depth markings)
- Niskin Bottle weight and messenger
- Borosilicate sampling bottles with glass stoppers
- Tygon tubing
- Mercuric chloride ( $\text{HgCl}_2$ )
- 200  $\mu\text{L}$  pipette and pipette tips
- Apiezon vacuum grease (decanted into a syringe for ease of use)
- Kimwipes
- Nitrile gloves
- Safety glasses (or sunglasses)
- Stopper rubber band and clamp
- Water sampling data sheet

### WAYPOINT AND METADATA COLLECTION

Use a GPS unit to collect a waypoint when the CTD downcast begins and the Niskin Bottle is triggered. Record all metadata on the water sampling data sheet.

### CTD CAST

1. Ensure that the CTD line is secured to the top of the CTD frame with at least 2 shackles.
2. Tie off the other end of the CTD line to the boat or clip off with a carabiner.
3. Flake CTD line on deck to ensure the CTD lowers smoothly.
4. Twist the CTD end cap to the ON position. Lower it over the side until the top of the frame is 1 meter below the surface of the water to begin the 1 minute soaking period, either holding the line or cleating off the line to maintain the CTD at soaking depth.
5. After a 1 minute soak, un-cleat the CTD line if it was cleated and begin the CTD cast by pulling the CTD frame up until the top ring of the frame emerges from the water. Then

begin gradually lowering at a consistent rate, hand over hand, until the CTD gets to the target depth (using the markings on the line to estimate depth).

6. Once the target depth is reached, pull the CTD back on board. Once the CTD is back on board, twist the end cap to the OFF position. Stow securely for transit.

## WATER SAMPLE COLLECTION

1. Prime the Niskin Bottle by opening the stoppers at both ends and ensuring the petcock and the air bleed valve are closed.
2. Clip off the boat side of the Niskin line to the boat, and attach the weight to the Niskin end of the line.
3. Near the end of the CTD soaking time, lower the weight and primed Niskin bottle over the side so the surface of water is at the 1m black mark drawn on Niskin line.
4. Clip the messenger on to the line.
5. When the CTD begins its downcast, release the messenger to trigger the Niskin to close.
  - **NOTE:** Ensure that no air bubbles are trapped inside the Niskin and the bottle sits vertically in the water column before firing the messenger. If the Niskin does not close when the messenger is released, retrieve the messenger and try again.
6. Once the sample has been collected, pull the Niskin bottle back on board. The water sample should be processed promptly following collection.

## WATER SAMPLE PROCESSING

1. Remove a labeled borosilicate bottle and its stopper from the storage tote (discard the twist tie that prevents the stopper sticking). Attach the tygon tubing to the Niskin bottle nipple. Insert the tygon tubing into the bottle, open the valve to start the flow of water, and rinse the sample bottle three times with ~20 mL of seawater, dumping the water between rinses. Gently swirl the water around the inside of the bottle during each rinse.
2. After rinsing, fill the sample bottle. Ensure that the tygon tube is placed at the bottom of the bottle, and allow seawater to and overflow the bottle for at least two complete flushings. To do so, start the collection and count how long it takes for the bottle to overflow and then allow that to occur for 2x the required fill time (e.g., if the bottle fills in 20 seconds, allow the water to flush the bottle for 40 seconds).
  - **NOTE:** Care should be given to ensure that smooth water flow into the bottle is maintained and that no bubbles are created during the dispensing of sample. Any bubbles introduced to the sample can alter the amount of dissolved inorganic carbon in the sample.
3. After the appropriate flushing time, shut off the Niskin valve to stop the water flow, while at the same time ensuring the tygon tubing doesn't come off the bottom of the sample

bottle. Once the flow is shut off, pinch the tubing and in one motion remove it from the bottle. This "pinch and remove" action with the tubing should establish a consistent head-space in all the sample bottles. The meniscus of the sample should be about 1 cm below the neck.

4. Once the proper head space is established, gently dry the inside of the bottle neck with a Kimwipe.
5. Open the saturated mercuric chloride ( $\text{HgCl}_2$ ) solution bottle. Using a fixed-volume pipette, dispense 200ul of  $\text{HgCl}_2$  to the sample bottle. Close the mercuric chloride bottle immediately after use.
  - **NOTE:** Supersaturated mercuric chloride solution is extremely dangerous; use the utmost caution when dealing with the chemical. All personnel working with it are required to wear eye protection. The mercuric chloride handler is also required to wear disposable nitrile gloves. In the event of contact with any part of the body, wash the area profusely. If contact is made with eyes, abort operations, rinse continuously with fresh water (or salt if fresh has run out).
  - **NOTE:** If the pipette tip is accidentally expelled into the sample bottle, do not attempt to remove it.
6. Use the syringe containing Apiezon vacuum grease to make 4 vertical "stripes" of grease on the clean, dry stopper. Insert the greased stopper until fully seated in the bottle, then twist until the grease completely seals the bottle contents. The vertical stripes of grease allow for air to escape the bottle neck while the stopper is being inserted. Having the stopper clean/dry ensures that other than sample water isn't introduced into the bottle. Twisting the stopper once it has been fully seated into the neck of the bottle ensures a smooth distribution of grease and an airtight seal.
7. Use the rubber band and plastic collar to lock down the stopper. Slide the plastic collar in between the two sides of the flattened rubber band, then pull the rubber band through the middle of the plastic collar so that the rubber band forms a U-shape. Position the rubber band over the top of the glass stopper, then pull down to place the plastic collar around the bottle neck. Tighten the collar so that the stopper is held firmly in place.
8. Once secured, invert the bottle 1-2x to mix the mercuric chloride. Secure the sample bottle in the field container.
9. Complete data sheet including site name, waypoint name (default), UTC date and time, latitude and longitude, sample depth and bottle number.
10. Samples should be stored in the storage totes indoors (preferably in air conditioning) and out of direct sunlight.

