DEPARTMENT OF ECOLOGY
State of Washington

# Standard Operating Procedure EAP025, Version 2.4

## Seawater Sampling

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# Purpose of this Document

The Washington State Department of Ecology develops Standard Operating Procedures (SOPs) to document agency practices related to sampling, field and laboratory analysis, and other aspects of the agency's technical operations.

# Publication Information

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Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

## SOP Revision History

| Revision Date | Revision History | Summary of Changes | Sections | Reviser(s) |
|---|---|---|---|---|
| 4/13/2020 | 2.3 | Addition of alkalinity and dissolved inorganic carbon sampling, zooplankton, changes to Particulate sampling and DO sensor sampling. Removal of primary productivity and secchi. | all | Mya Keyzers |
| 4/24/2023 | 2.4 | Updated with current sampling procedures. Removed outdated procedures for fecal bacteria and dissolved oxygen sampling. | all | Holly Young |

## 1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Standard Operating Procedure (SOP) of seawater field sampling for the Marine Waters Long-term Monitoring Program. Sampling procedures are performed monthly during marine water quality surveys conducted by boat.

## 2.0 Applicability

2.1 This SOP should be followed for all seawater sampling activities involved in the Marine Waters Long-term Monitoring Program.

## 3.0 Definitions

3.1 Chlorophyll: Group of pigments that allows plants, including algae, to convert sunlight into organic compounds through the process of photosynthesis. Chlorophyll *a* is the predominant pigment type found in algae and phytoplankton and is used as a proxy for phytoplankton abundance.

3.2 Niskin Bottle: Water sampling bottle used to make sub-surface measurements of water. These are cylindrical plastic bottles with spring-loaded end caps, an air-vent valve at one end, and a dispensing stopcock at the other.

3.3 Nutrient Analysis: Measures the amount of dissolved inorganic nitrate ($NO_3$), nitrite ($NO_2$), phosphate ($PO_4$), silicate ($SiOH_4$), and ammonium ($NH_4$) levels in water. Nutrient availability is used as an indicator of water quality.

3.4 Salinity: The total amount of dissolved salts measured in grams per one kilogram of sea water or parts per thousand.

3.5 Dissolved Inorganic Carbon (DIC): The sum of inorganic carbon species in a solution including carbon dioxide ($CO_2$), carbonic acid ($H_2CO_3$), bicarbonate anion ($HCO_3^-$), and carbonate ($CO_3^{2-}$).

3.6 Alkalinity: The capacity of water to resist acidification determined by measuring the amount of carbonate and bicarbonate levels in the water by chemical titration.

3.7 Material Safety Data Sheet (MSDS): Provides both workers and emergency personnel with the proper procedures for handling or working with a particular substance. The MSDS includes information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill/leak procedures.

3.8 Zooplankton: Small and diverse aquatic organisms that drift with water currents and can be capable of weak swimming. Zooplankton migrate daily through the water column, residing in deep water during the day to avoid predation, then moving to shallow water at night to feed on phytoplankton. Zooplankton are a fundamental food source for many higher trophic level organisms and even other zooplankton.

## 4.0 Personnel Qualifications/Responsibilities

4.1 All laboratory staff must comply with the requirements of the EAP Safety Manual (Environmental Assessment Program 2021).

4.2 Field staff must be familiar with the specific standard operating procedures described for the water quality parameters in this document. Several water quality parameters have specific collection and processing procedures.

4.3 Field staff must complete boat safety training and demonstrate competency in deckhand skills to participate in field sampling operations.

4.4 The field lead directs sample collection and is knowledgeable of the project's Quality Assurance Monitoring Project Plan (QAMP) to ensure that credible and useable data are collected. Sampling procedures and objectives are communicated to field team prior to arriving at the sampling site.

4.5 The job classes typically performing these duties include Natural Resource Scientist 1 and Natural Resource Scientist 2.

## 5.0 Equipment, Reagents, and Supplies

5.1 General Equipment and Supplies: nitrile exam gloves, deionized water, sample coolers with ice, sample bottle organizers, digital field log, backup paper logs, Sharpies, blank sample sticker labels, chain of custody forms, Kimwipes, D and AA cell batteries, work cellphone, VHF waterproof radio

5.2 Dissolved Inorganic Carbon and Total Alkalinity Sampling Supplies

- 500 mL sterilized or autoclaved glass bottles obtained from Pacific Marine Environmental Laboratory (PMEL)
- Whatman 0.45 µm non-sterile polycap GW encapsulated in-line filter, 75 mm diameter
- Silicone sample tubing
- Rubber bands and stopper clasps
- Paint pen
- Apiezon® L stopper grease
- Mercuric chloride ($HgCl_2$) prepared per SOP EAP028 (Coleman and Young, 2022). Highly toxic with carcinogenic and teratogenic hazards if exposed through physical contact.

5.3 Salinity Sampling Supplies

- Brown 125 mL polyethylene bottles

5.4 Dissolved Nutrient Sampling Supplies

- 60 mL narrow-mouthed, seawater-aged polyethylene bottles, obtained from the University of Washington's Marine Chemistry Lab
- 0.45 µm syringe filters, sterile cellulose acetate
- 60 mL plastic syringes
- Nutrient bottle dispenser

5.5 Particulate Sampling Supplies

- 1 L wide-mouth brown polyethylene bottles for both particulate organic carbon and particulate nitrogen samples provided by Manchester Ecology Lab (MEL)
- 125 mL wide-mouth clear HDPE polyethylene bottles with 1:1 hydrochloric acid preservative for total organic carbon. Chemical preservative is prepared by MEL and has corrosive hazards if exposed by physical contact or fumes.
- 125 mL wide-mouth clear HDPE polyethylene bottles with 1:1 sulfuric acid preservative for total nitrogen. Chemical preservative is prepared by MEL and has corrosive hazards if exposed by physical contact or fumes.
- Laboratory Analyses Required (LAR) Form
- Waterproof vinyl stickers with printed sample information

5.6 Chlorophyll *a* Sampling Supplies

- Brown 65 mL polyethylene bottles
- Chlorophyll *a* bottle dispenser

5.7 Zooplankton Sampling Supplies

- Ring net, 60 cm diameter, 200 µm mesh with attached cod end
- Flow meter
- Neutrally-buffered formalin in a squeeze bottle with measured reservoir dispenser. Reagent is prepared by staff in the Keister lab at University of Washington (Keister 2016). Hazards include corrosivity, carcinogenicity, and teratogenicity if exposed through physical contact or fumes.
- 20 lb weight with carabiner
- Handheld sprayer (Spray Doc)
- Sieve, 200 µm or smaller
- Paper field logs
- Various sizes of clear sample jars
- Squirt bottle
- Electrical tape

## 6.0 Summary of Procedure

6.1 Dissolved Inorganic Carbon (DIC) and Total Alkalinity (TA)

6.1.1 Collection of seawater must be done as soon as possible after opening the Niskin, preferably before other samples have been drawn to prevent contamination by atmospheric oxygen.

6.1.2 Using the Paint Pen provided by NOAA PMEL, write the PMEL Sequential Bottle ID and Date of Sample Collection on the 500 mL borosilicate glass DIC-TA sample bottles. The format of the PMEL Sequential Bottle IDs is ECY-###.

6.1.3 Connect the silicone tubing to the intake side of a Whatman encapsulated in-line filter and another piece of tubing to the outflow side.

6.1.4 Connect the silicone tubing to the Niskin stopcock, remove the white vent plug on the filter. Start the water flow, let the water run while agitating the filter to release any air bubbles. Pinch the silicone tubing until all the air bubbles are cleared and cap or cover the filter's air vent.

6.1.5 To conserve water, rinse and collect the paired salinity sample with the filter flushing water.

6.1.6 After salinity sample is collected or 10 seconds have elapsed since an air bubble passed through, place the silicone tubing until it is flush with the bottom of the 500 mL flask. With the water flowing, swish the water around and then invert to empty the water; repeat 3 times to rinse the bottle.

6.1.7 After the third rinse, fill the bottle and let it overflow one and a half times its total volume or about 20 additional seconds.

6.1.8 Bend and pinch the tubing and remove it from the sample bottle, then disconnect tubing from Niskin vent and close Niskin.

6.1.9 Use 1-10 mL pipette to remove 5 mL of sample to create head space for the glass stopper.

6.1.10 Carefully dry the stopper and bottle neck with a paper towel. Using the syringe of Apiezon L grease included with the DIC-TA sampling supplies, apply a thin layer of grease around the bottom of each bottle stopper.

6.1.11 Insert greased stopper into the neck of the DIC-TA sample bottle and fully rotate the stopper both clockwise and counterclockwise to create an airtight seal that will prevent gas exchange between the water sample and ambient atmosphere around it.

6.1.12 Seal bottle with a rubber band and collar per Figure 1. Place the whole collar through the middle of the rubber band (A). Pull both sides of the rubber band through the middle of the collar (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 (C). Be sure to pull the collar down so that it is below the neck of the bottle (D).

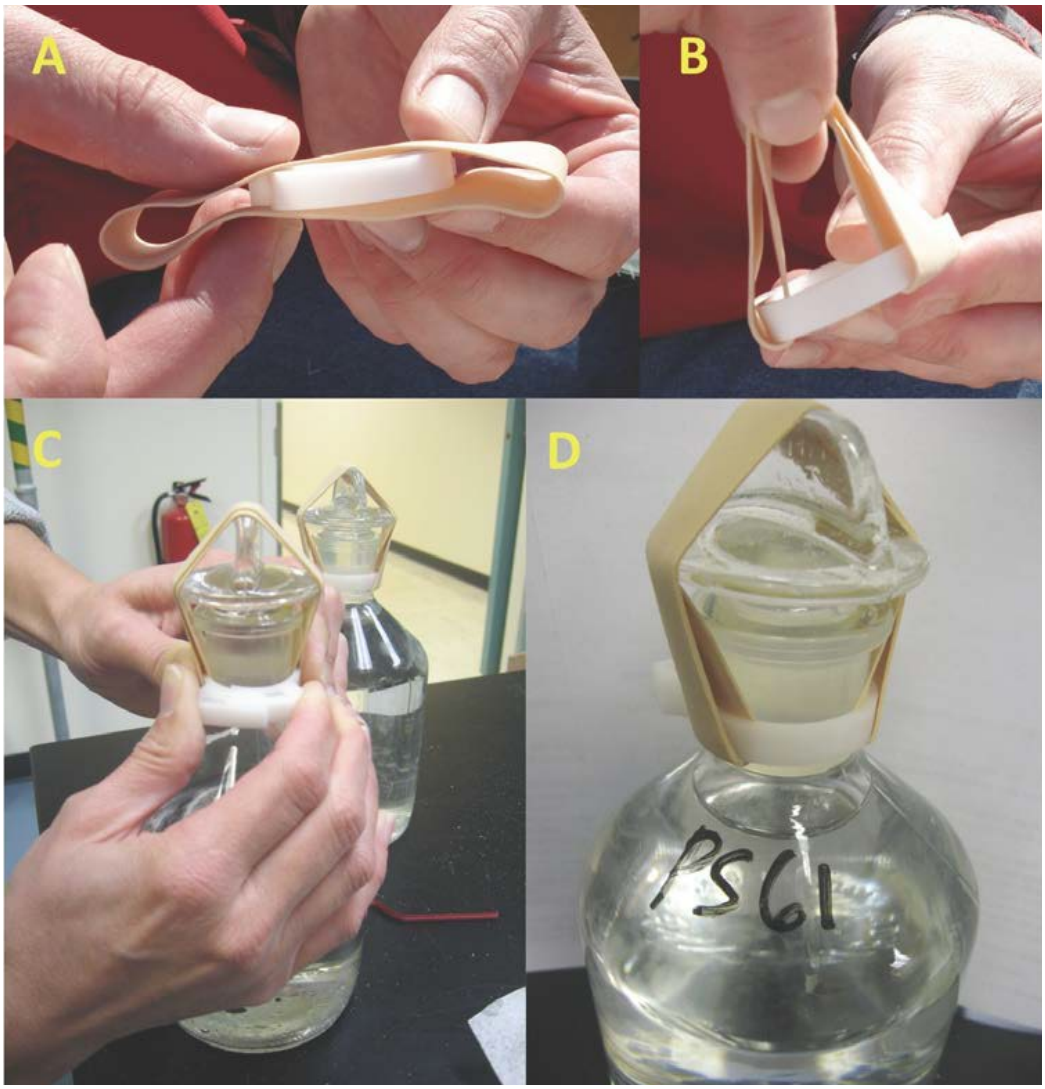


Figure 1. Procedures for sealing DIC-TA bottles usings rubber bands and plastic collars.

6.1.13 Place the bottles in a cooler with ice.

6.1.14 Back at the lab, using PPE (long-sleeves, gloves, and goggles), carefully pipette in 200 µL of mercuric chloride to each sample. For safety reasons, mercuric chloride will only be handled by the field lead and will always be transported in tertiary containment.

6.1.15 Reapply grease as needed and reseal DIC-TA sample bottle with rubber band and white collar.

6.1.16 Invert the sample several times to mix the mercuric chloride. The same pipette tip may be used to dispense the mercuric chloride since it does not touch the sample.

6.1.17 Dispose of pipette tip, gloves, and other contaminated materials properly.

6.1.18 Store samples in PMEL labeled totes in the walk-in cooler.

6.1.19 Fill out the chain of custody form. Samples should be delivered to PMEL within six months of collection (Dickson 2007).

6.2 Salinity

6.2.1 Salinity samples are collected from Niskin bottles at various depths and locations alongside the DIC-TA samples. When taking a new sample, rinse bottle three times with water from the Niskin. Fill the bottle two-thirds to completely full while purging air bubbles from the filter and tubing. Samples are stored in a temperature-controlled laboratory and analyzed in house per SOP EAP053 (Coleman 2022). Additionally, 10 salinity samples are analyzed by the University of Washington Marine Chemistry Laboratory per Grasshoff et al. 1999 methods.

6.3 Marine Dissolved Inorganic Nutrient Sampling

6.3.1 Seawater for nutrient analyses is collected from Niskin bottles after the DIC-TA. Sample bottles are 60 mL polyethylene bottles, obtained from the University of Washington Marine Chemistry Lab. Water samples are collected into 60 mL syringes with 0.45 µm SFCA syringe filters attached to them. The syringe barrel and plunger are rinsed three times with sample water before filtering sample. Sample bottles are rinsed three times with approximately 5-10 mL of clean filtrate for each rinse. The bottles are then filled about three-quarters full (35-40 mL) so that there is room for the sample to expand when frozen. Samples are stored frozen in the dark and analyzed within three months. Analysis is completed at the University of Washington Marine Chemistry Laboratory, according to the methods of Armstrong et al. 1967 (nitrate, nitrite, and silicate), Slawyk & MacIsaac 1972 (ammonium) and Bernhardt and Wilhelms 1967 (orthophosphate).

Figure 2. Particulate, chlorophyll *a*, and nutrient bottles in sample bins on boat deck.

6.4 Total Organic Carbon

6.4.1 Water samples are collected from Niskin bottles into 125 mL wide mouth clear HDPE polyethylene bottles with preservative 1:1 hydrochloric acid (HCl). Place the preprinted vinyl label on the bottle, ensuring it matches the field form information, and label with sampling date. Without rinsing, fill 2/3 full. Place in a cooler with ice. Fill out the Laboratory Analyses Required (LARS) sheet. Back at the lab, transfer all the samples to a cooler with blue ice and store at 4 °C in the walk-in cooler.

6.5 Total Nitrogen

6.5.1 Water samples are collected from Niskin bottles into 125 mL wide mouth clear HDPE polyethylene bottles with preservative 1:1 sulfuric acid ($H_2SO_4$). Place the preprinted vinyl label on the bottle, ensuring it matches the field form information, and label with sampling date. Without rinsing, fill 2/3 full. Place in a cooler with ice. Fill out the LARS sheet. Back at the lab, transfer all the samples to a cooler with blue ice and store at 4 °C in the walk-in cooler.

6.6 Particulate Organic Carbon/Nitrogen Sampling

6.6.1 Water samples are collected from Niskin bottles into clean 1 L amber polyethylene bottles provided by MEL. Place the preprinted vinyl label on the bottle, ensuring it matches the field form information, and label with sampling date. Without rinsing, completely fill to the neck of the bottle. Place in a cooler with ice. Fill out the LARS sheet. Back at the lab, transfer all the samples to a cooler with blue ice and store at 4 °C in the walk-in cooler.

Figure 3. Particulate sample bottles (organic carbon and nitrogen, total organic carbon and total nitrogen) in coolers for transit.

6.7 Chlorophyll *a* Sampling

6.7.1 Water samples are collected from Niskin bottles into clean amber 65 mL polyethylene bottles. Sample bottles should be rinsed three times with sample water, and filled until there is a positive meniscus. While filling, the stream of water should hit the neck of the bottle so that the water flows down the sides, thus minimizing shearing force on the phytoplankton cells.

6.7.2 Protect samples from heat and light to prevent degradation of the chlorophyll *a*. Samples should be filtered as soon as possible and can be temporarily stored in a cooler with ice or refrigerated.

Figure 4. Chlorophyll *a* and nutrient bottles in coolers for transit.

6.8 Zooplankton Sampling

6.8.1 Connect winch line to net and attach weight(s) to cod end. Check equipment for holes, tangles, and loose fittings.

6.8.2 Reset the flow meter dials to zero.

6.8.3 Deploy the net at 0.5 m/sec wire speed to desired depth watching the line angle. Account for additional line out during deployment if angle is greater than 0°.

6.8.4 Retrieve the net immediately at 0.5 m/sec and watch the line angle.

6.8.5 Visually check that the flow meter is spinning as it approaches the surface. If it is not spinning, this indicates a net clog, slow retrieval rate, or damaged flow meter.

6.8.6 Retrieve the net immediately upon reaching the surface (do not let it linger just below surface), taking care not to let the flow meter spin in the wind.

6.8.7 Record deployment information and flow meter revolutions on the field log.

6.8.8 Rinse the outside of the net using a seawater hose to concentrate the sample in the cod end. Start with a gentle rinse to protect delicate zooplankton. Pay special attention to seams that might catch organisms. Unhook the cod end, being careful that it is not overfilled. Strain the contents of the bucket through a sieve to concentrate.

6.8.9 Pour and thoroughly rinse contents into a sample jar with a squirt bottle, using a funnel if necessary.

6.8.10 Use the smallest sample jar possible. If the biomass is thick, use a larger jar or split into two jars. Leave enough room for formalin preservative before bringing sample up to volume.

6.8.11 Prepare to dispense formalin by wearing gloves and goggles and having the sample in secondary containment outside on the deck of the boat.

6.8.12 Preserve the sample using neutrally buffered formalin, adding enough to make the final sample concentration ~5% formalin. Squeeze the appropriate amount into the measured reservoir dispenser.

6.8.13 Fill sample jar to the bottom of the threads with seawater to prevent dehydration. Close tightly and swirl to mix.

6.8.14 Label the jar using a Sharpie with this format: Salish Sea Marine Survival Project (SSMSP), group, date, time, station, type of tow, mesh size, depth of tow, and flow meter reading. Wrap lid with electrical tape to seal the jar and store in the dark at ambient temperature.

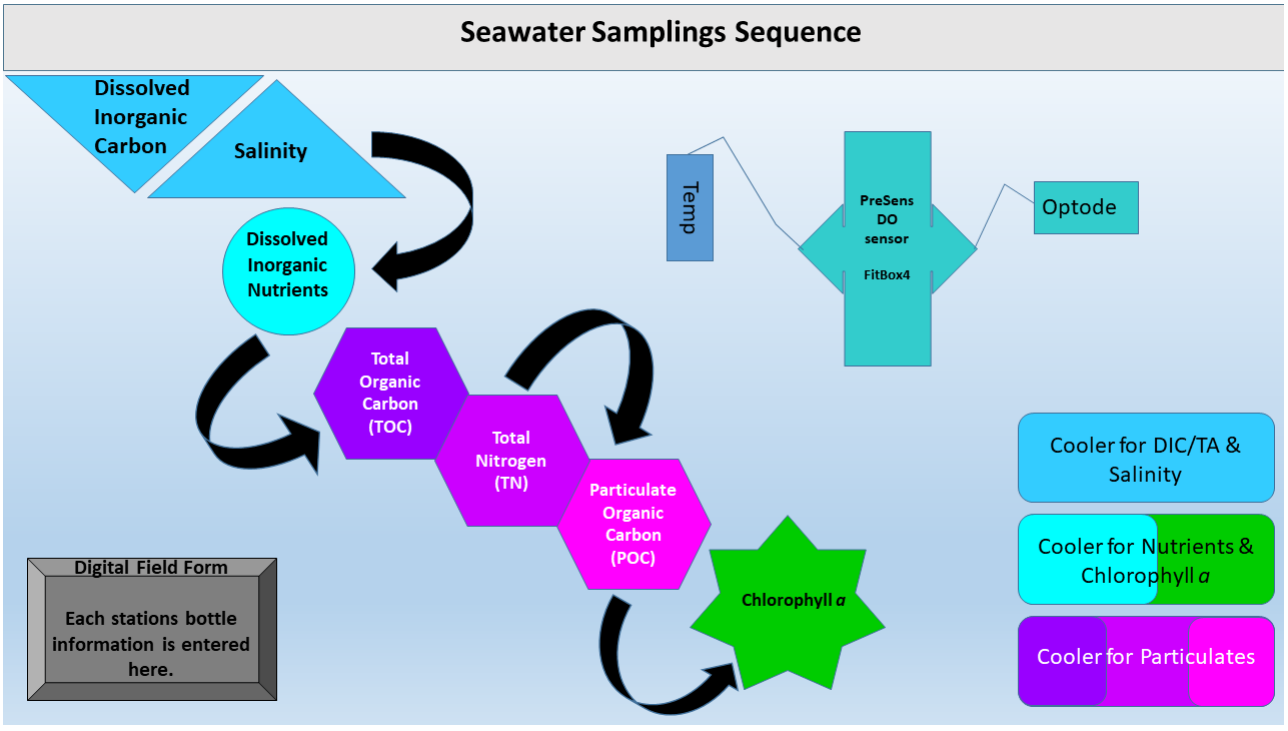


Figure 5. Flowchart of seawater collection sequence by sample type including coolers, digital forms, and handheld dissolved oxygen sensor.

## 7.0 Records Management

7.1 Site observations, comments, and sample bottle number IDs are recorded and saved in digital field forms. During post processing at the lab, physical bottles are cross checked against field form counts. Additionally, bottle IDs are recorded on chain of custody paper forms. Field forms are uploaded to the database through the Marine Waters Office Editor Application.

## 8.0 Quality Control and Quality Assurance

8.1 Replicate samples consisting of two samples collected from the same Niskin bottle and/or the same depth should be included at the discretion of the project lead. These samples will estimate the total random variability (precision) of individual samples.

## 9.0 Safety

9.1 Follow general procedures for safety found in the EAP Safety Manual, paying particular attention to those sections devoted to working from boats and the section on Marine Flights.

9.2 Any chemicals used in the field must have an MSDS and any additional handling information in the route binder.

9.3 Prior to boat work, all staff need to pass the EAP Personal Qualification Standards for the R/V Skookum.

9.4 Gloves, protective clothing, and safety glasses will be worn when handling formalin, mercuric chloride, hydrochloric acid, and sulfuric acid in the fume hood or field (outside only). Hazards include toxicity, carcinogenicity, and teratogenicity through physical and respiratory exposure.

## 10.0 References


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