

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Seawater Sampling

Version 1.0

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## Environmental Assessment Program

### Standard Operating Procedure for Seawater Sampling

#### **1.0 Purpose and Scope**

1.1 This Standard Operating Procedure (SOP) is for seawater sampling performed at long-term marine waters monitoring stations aboard marine flight surveys, as well as for ship-board sampling.

#### **2.0 Applicability**

2.1 This SOP should be followed for all seawater sampling activities performed by the Coastal and Estuarine Assessment Unit.

#### **3.0 Definitions**

3.1 **Chlorophyll:** Pigment that allows plants, including algae, to convert sunlight into organic compounds in the process of photosynthesis. Chlorophyll *a* is the predominant type found in algae and phytoplankton, and its abundance is a good indicator of the amount of algae present. In some cases, it is used as an indicator of water quality as high levels can indicate the presence of algae blooms. These blooms may be caused by excessive nutrient levels and can lead to depleted dissolved oxygen levels.

3.2 **Dissolved Oxygen (DO):** The amount of gaseous oxygen (O<sub>2</sub>) dissolved in water. Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement), and as a product of photosynthesis. Adequate DO levels are necessary to support aerobic life forms and oxygen is an element necessary to all forms of life. DO levels are used as an indicator of water quality.

3.3 **Fecal Coliform:** Bacteria that live in waste material or feces of warm-blooded animals and humans. When fecal coliform bacteria are present in high numbers in a water sample, it may indicate that the water may have received fecal matter from one source or another, thus is used as an indicator of water quality.

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

- 3.5 Nutrient Analysis: Measures the amount of nitrate, nitrite, phosphate, silicate and ammonium levels in water. Excess nutrients may lead to significant water quality problems including harmful algal blooms, hypoxia, declines in wildlife and wildlife habitat, and have been linked to increases in human pathogens. Nutrient measurements are used as an indicator of water quality.
- 3.6 Salinity: Salinity is the total amount of dissolved material in grams in one kilogram of sea water. Samples are collected to calibrate and check conductivity measurements made by the CTD.
- 3.7 Secchi Disk: Measures transparency of the water using an 8-inch diameter black and white disk attached to a rope. The rope is marked at 0.5 meter intervals for easy determination of depth.
- 3.8 Secchi Depth: Depth in the water at which the disk is no longer visible. It is usually the average between the depth at which the disk is no longer visible when it is lowered into the water and the depth at which it is again visible as the disk is raised. The secchi depth can be used to calculate the amount of colored substances (i.e., phytoplankton, algae, and detritus) in the water. Changes can be caused by sediment runoff from land or increased phytoplankton populations. Changes in Secchi depth over time are used as an indicator of water quality.
- 3.9 MSDS – Material Safety Data Sheets provides both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS's include 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.

#### **4.0 Personnel Qualifications/Responsibilities**

- 4.1 All field staff must comply with the requirements of the EA Safety Manual (EA Program, 2006).
- 4.2 All field staff must be familiar with other standard procedures described for water quality parameters in this document. Several water quality parameters have special sample pre-treatment, filtering, post-treatment, and collection procedures applicable to this document.
- 4.3 The Field Lead directing sample collection must be knowledgeable of all aspects of project's Quality Assurance Project Plan (QAPP) to ensure that credible and useable data are collected. All field staff should be briefed by the Field Lead or Project Manager on the sampling goals and objectives prior to arriving to the site.

## **5.0 Equipment, Reagents, and Supplies**

### 5.1 General Equipment and Supplies

- 5.1.1 Safety goggles
- 5.1.2 Nitrile exam gloves
- 5.1.3 De-ionized water
- 5.1.4 Sample coolers with ice
- 5.1.5 Field log notebook and pens
- 5.1.6 Sample tags
- 5.1.7 Kimwipes
- 5.1.8 Batteries (D cells and AA cells)

### 5.2 Dissolved Oxygen Sampling Supplies

- 5.2.1 Wooden boxes each containing 24 125 ml glass flasks and stoppers for collection of dissolved oxygen samples
- 5.2.2 Tygon tubing
- 5.2.3 3 M Manganese chloride ( $\text{MnCl}_2$ ) (obtained from the University of Washington's Marine Chemistry Lab). This chemical is stable for 2 years when stored in sealed plastic bottles and kept in the dark. The MSDS can be found at <http://www.jtbaker.com/msds/englishhtml/M0767.htm>.
- 5.2.4 8 N Sodium hydroxide-sodium iodide sodium-azide (NaOH-NaI-Azide) (obtained from the University of Washington's Marine Chemistry Lab). This chemical is stable for 2 years when stored in sealed plastic bottles and kept in the dark. Sodium azide is a suspected carcinogen and should be treated with care. The MSDS's for NaOH, NaI and  $\text{NaN}_3$  can be found at <http://www.jtbaker.com/msds/englishhtml/S4034.htm>, <http://www.jtbaker.com/msds/englishhtml/S4202.htm> and <http://www.jtbaker.com/msds/englishhtml/S2906.htm>.
- 5.2.5 De-ionized water (18 megohm)
- 5.2.6 Bottle-top dispensing pipettes for safely dispensing chemicals

- 5.3 Salinity Sampling Supplies
- 5.3.1 Brown 125 ml polyethylene bottles with previous sample still in them
- 5.4 Chlorophyll a Sampling Supplies
- 5.4.1 Brown 65 ml polyethylene bottles
- 5.4.2 Certified ACS grade 90% acetone. Acetone is not known to be carcinogenic or teratogenic, but it can cause defatting of skin tissues on contact. The MSDS may be found at <http://www.jtbaker.com/msds/englishhtml/A0446.htm>. Dilute to 90% with de-ionized water. Follow the Reagent Preparation SOP to make 90% acetone.
- 5.4.3 25 mm 0.45um Whatman glass fiber filters (GF/F)
- 5.4.4 Filter forceps – stainless steel, straight, flat, smooth tip
- 5.4.5 Gast oil-less vacuum pump
- 5.4.6 Polycarbonate in-line filter holders and manifold
- 5.4.7 12 ml clear glass centrifuge tubes
- 5.4.8 Aluminum foil
- 5.5 Dissolved Organic Carbon and Particulate Carbon/Nitrogen Sampling Supplies
- 5.5.1 1 L polyethylene bottles
- 5.5.2 pre-muffled 25 mm 0.45 um Whatman glass fiber filters (GF/F)
- 5.5.3 Filter forceps – stainless steel, straight, flat, smooth tip
- 5.5.4 Polycarbonate in-line filter holders
- 5.5.5 1 L polyethylene side-arm flasks
- 5.5.6 Tygon tubing
- 5.5.7 Petri dishes (obtained from the University of Washington’s Marine Chemistry Lab)
- 5.5.8 40 ml sterile glass vials (obtained from the University of Washington’s Marine Chemistry Lab)

- 5.6 Marine Dissolved Nutrient Sampling Supplies
- 5.6.1 60 ml narrow-mouthed polyethylene bottles (obtained from the University of Washington's Marine Chemistry Lab)
- 5.6.2 25 mm sterile Nalgene syringe filters
- 5.6.3 60 ml plastic syringes
- 5.7 Total Nitrogen / Total Phosphorus Sampling Supplies
- 5.7.1 60 ml wide-mouthed polyethylene bottles
- 5.7.2 25 mm sterile Nalgene syringe filters
- 5.7.3 60 ml plastic syringes
- 5.7.4 20 ml graduated cylinders
- 5.8 Alkalinity Sampling Supplies
- 5.8.1 250 ml polyethylene bottles obtained from the Manchester Environmental Laboratory (MEL)
- 5.8.2 Sample tags
- 5.9 Phytoplankton Species and Biovolume Sampling Supplies
- 5.9.1 120 ml glass jars with Teflon sealed lids
- 5.9.2 7.4% Formalin. Dilute 20 mL of 37% formaldehyde into de-ionized water for a final volume of 100 mL. Formaldehyde is a known carcinogen, so extreme care must be taken when handling this chemical. The MSDS for 37% formaldehyde may be found at <http://www.jtbaker.com/msds/englishhtml/F5522.htm>.
- 5.10 Primary Productivity Sampling
- 5.10.1 125 ml square polyethylene bottles and wooden cases
- 5.10.2 Secchi disc
- 5.10.3 Sodium bicarbonate –  $^{14}\text{C}$  ( $\text{NaH}^{14}\text{CO}_3$ ) ( $^{14}\text{C}$  isotope). This is a buffered aqueous solution that has a concentration of 2-10 mCi per mmol. This is a radioactive isotope. May be carcinogenic and may cause genetic mutation. The MSDS may be found at <http://www.sigmaaldrich.com/cgi-bin/hsrun/Suite7/Suite/HAHTpage/Suite.HsSigmaAdvancedSearch.formAction>.

- 5.10.4 Ammonium chloride (NH<sub>4</sub>Cl) and Potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>). The nutrient stock solution is prepared by combining 1.348 g/L NH<sub>4</sub>Cl and 0.343 g/L KH<sub>2</sub>PO<sub>4</sub>. Adding 200 μL of this solution to a 168 mL sample gives a final concentration of 30 μM NH<sub>4</sub><sup>+</sup> and 30 μM PO<sub>4</sub><sup>-</sup>. This solution may be kept in the cold and dark for 2 years. The MSDS's for these chemicals may be found at <http://www.jtbaker.com/msds/englishhtml/A5724.htm> and <http://www.jtbaker.com/msds/englishhtml/P6038.htm>.
- 5.10.5 Flow-through incubator
- 5.10.6 Screen bags
- 5.10.7 LI-COR Biosciences LI-192 Underwater Quantum Sensor with the LI-1400 DataLogger
- 5.10.8 Pipettes
- 5.10.9 Polycarbonate in-line filter holders and manifold
- 5.10.10 Vacuum pump
- 5.10.11 Bench covers
- 5.11 Fecal Coliform Sampling Supplies
- 5.11.1 Sterile 250 ml polyethylene bottles obtained from MEL
- 5.11.2 metal fecal bottle samplers
- 5.11.3 Sample tags

## **6.0 Summary of Procedure**

- 6.1 Dissolved Oxygen Sampling
  - 6.1.1 Collection of water at sea (from the Niskin bottle) must be done as soon as possible after opening the Niskin, preferably before any other samples have been drawn. This is necessary to minimize exchange of oxygen with the head space in the Niskin which typically results in contamination by atmospheric oxygen.
  - 6.1.2 The oxygen samples are drawn into the individually numbered and calibrated BOD bottles. It is imperative that the bottle and stopper is a matched pair. One sample is drawn from each Niskin.
  - 6.1.3 When obtaining the water sample, great care is taken to avoid introducing air bubbles into the sample. A 30–50 cm length of Tygon tubing is connected to the Niskin bottle spout. The end of the tube is elevated before the spout is opened to

prevent the trapping of bubbles in the tube. With the water flowing, the tube is checked for trapped air bubbles. If bubbles are found, the tube is squeezed to move the bubbles out the end of the tube. With the water flowing, the tube is placed in the bottom of the horizontally held BOD bottle in order to rinse the sides of the flask and the stopper. The bottle is turned upright and the side of the bottle tapped to ensure that no air bubbles adhere to the bottle walls. Three volumes of water are allowed to overflow from the bottle. The tube is then slowly withdrawn from the bottle while water is still flowing.

6.1.4 Immediately after obtaining the seawater sample, the following reagents are introduced into the filled BOD bottles by submerging the tip of a pipette or automatic dispenser well into the sample: 1 ml of manganese chloride, followed by 1 ml of sodium iodide-sodium hydroxide solution.

6.1.5 The stopper is carefully placed in the bottle ensuring that no bubbles are trapped inside. The bottle is vigorously shaken, then reshaken roughly 20 minutes later when the precipitate has settled to the bottom of the bottle. The neck is then sealed with deionized water. The sample should be analyzed after a period of at least 6-8 hours but within 5 days. The sample is stable at this stage. Analysis is completed at the EAP Operation Center's Marine Lab, according to the methods of Carpenter, 1966.

## 6.2 Salinity Sampling

6.2.1 Salinity samples are collected from Niskin bottles at all depths. These samples are collected after the oxygen samples have been drawn. The bottles used are 125 ml polyethylene bottles. The remaining sample from the previous use is left in the bottles between uses to prevent salt crystal buildup from evaporation and to maintain equilibrium with the bottle. When taking a new sample, the old water is discarded and the bottle is rinsed three times with water from the new sample. It is then filled 2/3 full with sample. The cap is then replaced and firmly tightened. These samples are stored in a temperature controlled laboratory for later analysis. Analysis is completed at the University of Washington Marine Chemistry Laboratory, according to the methods of Grasshoff et al., 1999.

## 6.3 Chlorophyll *a* Sampling

6.3.1 Water samples are collected from Niskin bottles into clean brown 65 ml polyethylene bottles. Samples should be protected from heat and light to prevent degradation of the chlorophyll. Sample bottles should be rinsed three times with sample water. 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. Samples should be filtered as soon as possible, and can be temporarily stored in a cooler with ice or refrigerated (not frozen). Samples are filtered through 25 mm GF/F filters using polycarbonate in-line filter holders (Gelman) and a vacuum pressure of 5-7 psi. The sample bottle is rinsed with a squirt of filtered seawater and the rinse is added to the sample in the filter cup.

6.3.2 Filters are immediately placed into a glass centrifuge tube and 10 ml of 90% acetone is added, submerging the filter. The centrifuge tube is wrapped in foil and stored in the fridge or freezer for at least 2 hours before fluorometric analysis is performed. Samples are stable in the freezer for up to one month before analysis. Analysis is completed at the EAP Operation Center's Marine Lab, according to the methods of the EPA, 1977.

#### 6.4 Dissolved Organic Carbon and Particulate Carbon/Nitrogen Sampling

6.4.1 Water samples are collected from Niskin bottles into clean 1L polyethylene bottles, rinsed three times with sample water. Samples are then filtered through 25 mm GF/F filters using polycarbonate in-line filter holders (Gelman) and a vacuum pressure of 5-7 psi. The volume of water filtered varies, depending on the sample. A sufficient volume of water is used to ensure a 'dark' color on the filter pad, up to 1L (volume is recorded for further use). The filter pad is then placed in a labeled petri dish and stored in the freezer until analyzed. This is the particulate carbon/nitrogen fraction of the sample. These samples are analyzed on an Exeter Analytical, Inc. (2005) Model 440 CHN/O/S Elemental Analyzer (see references below). Dissolved organic carbon samples are obtained by measuring 20-30 ml of the filtrate and transferring it to a 40 ml glass DOC vial. Samples are stored in the freezer until analyzed. The dissolved organic carbon samples are analyzed on a Shimadzu TOC-Vcsh carbon analyzer, using the high temperature catalytic oxidation method (HTCO) and measured on a non-dispersive infrared (NDIR) detector. The samples are acidified with 6N HCl, sparged and injected into the system. Analysis is completed at the University of Washington Marine Chemistry Laboratory, according to the methods of Grasshoff et al., 1999.

#### 6.5 Marine Dissolved Nutrient Sampling

6.5.1 Water samples are collected from Niskin bottles after dissolved oxygen samples have been removed. Sample bottles are 60 ml polyethylene bottles, obtained from Kathy Kroglund at the University of Washington Marine Chemistry Lab. Water samples are collected into new or pre-acid-cleaned 60 ml syringes with 0.45  $\mu\text{m}$  SFCA syringe filters attached to them. The syringe barrel and plunger are rinsed 3 times with sample water before filtering sample. Sample bottles are rinsed 3 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 until analysis. Frozen samples should be analyzed within 3 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 & Wilhelms, 1967 (orthophosphate).

## 6.6 Total Nitrogen / Total Phosphorus Sampling

6.6.1 Water samples are collected from Niskin bottles into 60 ml polyethylene bottles. Both filtered and unfiltered water samples are collected. For unfiltered samples, water samples are collected directly into 20 ml graduated cylinders that have been rinsed 3 times with sample water. 20 ml of sample water is measured into the graduated cylinder and transferred to the sample bottle. Samples are stored on ice or in the freezer until analysis. For filtered samples, water samples are collected into new or pre-acid-cleaned 60 ml syringes with 0.45 µm SFCA syringe filter attached to them. The syringe is rinsed 3 times with sample water. 20 ml of sample water is filtered directly into the 60 ml sample bottle and stored on ice or in the freezer until analysis. Analysis is completed at the University of Washington Marine Chemistry Laboratory, according to the methods of Valderrama, 1981.

## 6.7 Alkalinity Sampling

6.7.1 Water samples are collected from Niskin bottles into clean 250 ml polyethylene bottles. Sample water is allowed to gently flow down the side of the bottle to minimize bubbles in the sample. The sample bottle is gently shaken while being filled to get ride of bubbles attached to the sides of the bottle. Samples are stored on ice for transfer to Ecology's Manchester Lab for analysis, according to the methods of Strickland & Parsons, 1968.

## 6.8 Phytoplankton Species and Biovolume Sampling

6.8.1 Water samples are collected from Niskin bottles into 120 ml glass jars with Teflon sealed lids. Approximately 100 ml of sample is dispensed into the glass jar and preserved in the field with 0.148% formalin (2 ml formalin in 100 ml seawater).

## 6.9 Primary Productivity Sampling

6.9.1 Water for primary productivity experiments comes from Niskin bottles filled at different depths in the water column. These depths are determined by first measuring light penetration in the water column using a secchi disc. The secchi depth is determined by measuring the depth of water at which the disc just disappears from sight. The secchi depth is used to calculate the depths for 100, 50, 25, 12, 6 and 1% light transmission levels. Nutrient and chlorophyll samples are also collected at all of the above light levels. Two different sets of three 125 ml polyethylene sample bottles (2 "lights" and 1 "dark") are filled with water from the Niskin bottle. Bottles and caps are rinsed 3 times with sample water and carefully filled to the brim by allowing the stream of water to hit the side of the bottle, thus minimizing turbulence and shearing of the cells. Bottles are kept in wooden boxes to minimize light exposure (2 boxes are inoculated per experiment).

6.9.2 A flow-through incubator is set up on the ship deck, filled with water and a terrestrial PAR sensor is attached to it. Samples are then inoculated with 1.2  $\mu\text{Ci}$  of  $^{14}\text{C}$  per bottle. One box of bottles is also inoculated with excess nutrients to test for nutrient limitation: 30  $\mu\text{M}$   $\text{NH}_4^+$  and 3  $\mu\text{M}$   $\text{PO}_4^{3-}$ . After all bottles are inoculated, dark bottles are placed at one end of the incubator and all 100% light bottles at the other. All other bottles are placed in the appropriate screen bag (4 bottles per bag) for their light level (50, 25, 12, 6 or 1%). The bottles are wedged together so they will not float around the incubator. Incubation start time is recorded and incubations run for 24 hours.

6.9.3  $T_0$  samples and  $T_0$  nutrient spike samples are taken to determine the ambient and enriched nutrient concentrations and are not inoculated with  $^{14}\text{C}$ . They are filtered immediately after the nutrient spike has been added. If filtration must be delayed, then the samples are not inoculated until just before filtration. The filtered nutrient spike samples are stored frozen until analysis. Analysis is completed at the University of Washington according to the methods of Parsons et al., 1984.

## 6.10 Fecal Sampling

6.10.1 Fecal samples are collected from the surface of the water using sterile 250 ml polyethylene bottles (obtained from Manchester Lab). The sample bottle is placed in a metal 'sampler' and lowered to the water surface. The bottle is filled nearly to the top with the surficial layer of water. The sample is then returned to the deck, capped and placed on ice until analysis. Analysis is completed at the Manchester Lab, according to the methods listed in MEL, 2005.

## 7.0 Records Management

7.1 Field logs must be completed for each station sampled and sample bottle numbers recorded for each parameter sampled.

## 8.0 Quality Control and Quality Assurance Section

8.1 Replicate samples: 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 *Environmental Assessment Program Safety Manual*, paying particular attention to those sections devoted to working from boats and the section on Marine Flights.

9.2 Gloves and safety glasses should be worn when handling formalin, MnCl<sub>2</sub>, NaOH-NAI-Azide and acetone.

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