1 POINT OF CONTACT

NAME: Peter Linderoth, Save the Sound / CFE, Water Quality Program Manager
ADDRESS: 545 Tompkins Ave, 3rd Floor, Mamaroneck, NY 10543
EMAIL: plinderoth@savethesound.org
PHONE: 914-263-6233

2 OBJECTIVE

Determine the concentration of chlorophyll $a$ in the surface water, 0.5 m below the surface, following guidelines of the Unified Water Study (UWS). Frequency of sampling and daily order of events are specified in the “UWS Sampling Plan SOP.”
3 DEFINITIONS AND ABBREVIATIONS

Embayment: A recess in a coastline or an indentation off a shoreline which forms a bay. In Long Island Sound, the names of embayments often include the words Harbor (27%), River (23%), Cove (19%), Bay (10%), Creek (10%), and Pond (7%); with a few including the names Brook, Gut, Inlet, or Lake.

Field Team: Person or group of people working together to sample a station.

Monitoring Group: The group conducting the field work.

Section: The reporting regions for the embayment report card. Each section must include a minimum of three stations. Sections will be assigned a unique name by the UWS; examples are included below.

<table>
<thead>
<tr>
<th>Number of Sections</th>
<th>1</th>
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<tbody>
<tr>
<td>Name of Sections</td>
<td>whole</td>
<td>inner</td>
<td>outer</td>
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<td>Abbreviations for Sections</td>
<td>W</td>
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Site: The whole embayment, as defined by the UWS list. Each site has a unique three letter code assigned by the UWS; for example, Little Neck Bay, NY is “LNE”.

SOP: Standard operating procedure; this document is a SOP.

Station: The location where samples are collected, identified by a GPS location.

UWS: Unified Water Study

UWS Coordinator: The person designated as the point of contact for the UWS.

UWS Scientific Advisor: Estuarine or water quality scientists designated as advisors to the UWS.

4 OVERVIEW

A water sample is collected, filtered, and analyzed at an analytical lab to determine the chlorophyll \( a \) concentration using the fluorometric technique (90% acetone extraction). Phytoplankton, the microscopic plant-like organisms living in the water, contain chlorophyll \( a \). Thus, chlorophyll \( a \) concentration provides a rough approximation of the amount of phytoplankton in the water column. This is considered a rough approximation because individual phytoplankton contain varying amounts of chlorophyll \( a \) based on species, size, and environmental factors.

At each station, a water sample is collected from 0.5 m below the surface. The sample may be held in the dark on ice for up to four hours before filtering. The water sample is filtered through a glass fiber filter with pore size of 0.7 \( \mu m \). Two replicates of the collected water are filtered. The filter is dried by passing air through the filter. The filter is stored in the dark on ice until it can be transferred to a freezer (within 12 hours of the initial sample collection).

Monitoring groups should consult and comply with any additional methods associated with the specific certified lab chosen.
5 SOURCES

These procedures are based on the EPA Volunteer Estuary Monitoring Manual (EPA, 2007) and follow methods used in the EPA National Coastal Assessment (EPA, 2001). The EPA Volunteer Estuary Monitoring Manual (EPA, 2007) provides a wealth of specific data for monitoring groups. All groups should refer to the EPA manual for specific guidance.


6 MATERIALS AND EQUIPMENT

- 500 mL (or larger) sample bottle, bottle must be opaque if not filtering immediately following collection. Bottle may be high density polyethylene plastic (HDPE), high density polycarbonate plastic (HDPC), or glass.
- Sampling pole with clearly marked 0.5 m measurement to ensure correct depth is sampled.
- Glass fiber filters (Whatman GF/F with nominal pore size of 0.7 μm), 2.5 cm diameter, (Fisher Scientific Catalog Number: 09-874-64; Whatman Number: 1825-025)
- Filter holders, 2.5 cm (preferred: Pall brand, 25 mm Easy Pressure Syringe Filter Holder, Delrin Plastic; second choice: Millipore Swinnex Filter Holder, 25 mm polypropylene with silicon gasket, Fisher Scientific Catalog Number: SX00 025 00; Millipore Number: SX0002500)
- 60 mL syringe
- Forceps for handling filters
- Blotting paper or unused coffee filter cut into strips
- Aluminum foil
- Airtight container
- Cooler
- ASTM type III (distilled or deionized) water (ASTM Type II or I are also acceptable); store in an opaque bottle which has only held pure water (for field blank)
7 METHODS

7.1 Preparation

- Prepare equipment and supplies according to the recommended sampling procedure of the laboratory where the samples will be analyzed. The lab may offer the opportunity to place filters directly into analysis vials.
- Check that field equipment is prepped and operational.
- Prepare labels for filters. The labels should include the UWS unique station ID, indicator of the date, and replicate ID ("a" or "b"). For example:
- Prep aluminum foil squares for projected number of samples (2 per station) plus extra.
- Load all available filter holders with filters. Ideally, you will have enough filter holders to last through the day (2 per station, plus 2 blanks). Bring enough filters to last the sampling day plus extra.

7.2 Field Collection and Processing

1. Collect water sample from 0.5 m below surface (see “Sampling Plan SOP” for details).
   - Rinse the sampling water bottle three times with sample water from the appropriate depth. To do this, remove the cap from the bottle, holding the bottle with the mouth down, lower it to 0.5 m below the surface. Invert the bottle so that air empties out of the bottle and the bottle fills with water. Place your hand or the sample bottle cap over the mouth and bring to the surface.
2. If filtering immediately, proceed to step 3.
   - Store opaque bottle on ice in a cooler.
   - Filter within 4 hours of sample collection.
3. If not prepped, load filters into the filter holder.
   - Preloading all filter holders with filters before the sampling event begins is strongly advised.
4. Rinse the 60 mL syringe with ~5 mL of sample water. Repeat for a total of three rinses.
5. Mix the sample well by inverting the sample bottle 5 times.
6. Fill the 60 mL syringe with sample water. Note the volume of water in the syringe – knowing the exact volume filtered is critical to analyzing the chlorophyll a concentration.
7. Connect the filter holder to the syringe.
8. Gently expel water through the filter. The pressure applied to the plunger of the syringe should be slow and steady. If you encounter more than mild resistance, the filter may be clogged (e.g. jellyfish, algae) or you may have filtered sufficient sample.
9. After filtering 60 mL, inspect the filter for color. If a visible light green or light brown color is present, there is sufficient material on the filter for analysis, skip to step 10.
   - If no color is visible, filter another 60 mL and re-inspect.
   - On some occasions, if chlorophyll \(\alpha\) levels are very high, filtering may become difficult after as little as 30 mL. If this occurs, and the filter is colored, stop filtering and proceed to step 10.

10. Recording the exact volume filtered is critical to analyzing the chlorophyll \(\alpha\) concentration. If you are uncertain of the volume filtered, discard the sample and start over.

11. Use the 60 mL syringe to dry the filter by expelling air through the filter.
   - With the syringe NOT attached to the filter holder, draw air into the syringe.
   - Attach syringe to holder and expel air forcefully through the filter. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times.
   - DO NOT draw air backwards through the filter. Syringe should be taken off holder each time plunger is drawn up.

12. Remove filter with forceps.
   - If forceps are dirty, wipe with a Kimwipe or rinse with distilled water.
   - Only touch the filter on the edges. You may use clean fingers to steady the filter by touching the edge (do not touch the green or brown part).
   - If the analytical lab has provided analysis vials, place the dried filter directly into a vial. Label with the UWS unique station ID, date, and replicate letter (“a” or “b”). Proceed to step 13.
   - If the filter will be stored in aluminum foil, fold filter in half with forceps and place it in an absorbent pad (e.g. blotting paper or a coffee filter). Wrap in aluminum foil by folding the foil around the filter. Label with the UWS unique station ID, date, and replicate letter (“a” or “b”). Proceed to step 13.

13. Filters should be placed in an airtight container and stored in a cooler on ice. Ice should surround the airtight container.

14. Perform a field blank at least once during a sampling event. Follow the procedures for sampling above, but use the ASTM type III (distilled or deionized) water (ASTM Type II or I are also acceptable) water brought out on the boat in place of the field water.

7.3 Sample Storage

15. Store samples in the freezer (-20°C). Frozen samples must be analyzed within 28 days.

7.4 Laboratory Analysis

16. Bring filters to partner lab for analysis. Frozen samples must be analyzed within 28 days.
8 TROUBLESHOOTING / HINTS

- Make sure filters are dry before storing.
- Gather field equipment the day prior to sampling. Check the field equipment in the morning, before you head out into the field.
- Always carry a copy of this SOP and the relevant parameter-specific SOPs.
- Print out the “quick sheets” for relevant SOPs to use as a reminder in the field. Do not laminate these as you will want to add notes. A plastic page-protector taped close can be used to keep these sheets dry.

9 DATA PROCESSING AND STORAGE

The UWS coordinator will be the custodian of the finalized data files. The UWS coordinator will maintain a database which includes the unique site codes, section codes, and station codes for the embayment. Each unique station code will be affiliated with the corresponding GPS for the station.

The monitoring group is responsible for obtaining data, entering data into the UWS data template, and delivering the data to the UWS coordinator.

The monitoring group is responsible for assuring that the correct unique station ID assigned by the UWS is properly matched with the local organizations station ID codes. Both codes (monitoring group’s station code and UWS unique station ID) will be entered into the data template, along with the GPS coordinates.

10 REFERENCES


11 Quick Sheet – Filtered Chlorophyll a

SAMPLE ONLY 0.5 m BELOW SURFACE

Recording the exact volume filtered is critical to analyzing the chlorophyll a concentration. If you are uncertain of the volume filtered, discard the sample and start over.

The labels should include the UWS unique station ID, replicate ID (“a” or “b”), and date. For example:

- blank: LNE a 6/8/17 & LNE b 6/8/17

Perform a field blank at least once during a sampling event. Follow the procedures for sampling above, but use the ASTM type III (distilled or deionized) water (ASTM Type II or I are also acceptable) brought out on the boat in place of the field water.

1. If not filtering immediately, store bottle on ice in a cooler; filter within 4 hours of sample collection.
2. Rinse the 60 mL syringe with ~5 mL of sample water. Repeat for a total of three rinses.
3. Mix the sample well by inverting the sample bottle 5 times. Fill the 60 mL syringe with sample water. **Note the volume of water in the syringe – knowing the exact volume filtered is critical to analyzing the chlorophyll a concentration.**
4. Gently expel sample water through the filter. The pressure applied to the plunger of the syringe should be slow and steady. If you encounter more than mild resistance, the filter may be clogged (e.g. jellyfish, algae) or you may have filtered sufficient sample.
   - After filtering 60 mL, inspect the filter for color. If a visible light green or light brown color is present, there is sufficient material on the filter for analysis. If no color is visible, filter another 60 mL and re-inspect. On some occasions, if chlorophyll a levels are very high, filtering may become difficult after as little as 30 mL.
5. **Recording the exact volume filtered is critical to analyzing the chlorophyll a concentration. If you are uncertain of the volume filtered, discard the sample and start over.**
6. Use the 60 mL syringe to dry the filter by expelling air through the filter.
   - Attach syringe to holder and expel air forcefully through the filter. Do this until no “mist” is aspirated from the filter holder, a minimum of 3 times. DO NOT draw air backwards through the filter. Syringe should be taken off holder each time plunger is drawn up.
7. Remove filter with forceps.
   - Only touch the filter on the edges. You may use clean fingers to steady the filter by touching the edge (do not touch the green part).
   - Place filter in analysis vial supplied by analytical lab – OR – fold filter in half with forceps, place in an absorbent pad (e.g. coffee filter), wrap in aluminum foil and apply the label.
8. Labeled, foil wrapped filters should be placed in an airtight container and stored in a cooler on ice. Ice should surround the airtight container.
9. Store samples in the freezer (-20°C). Bring filters to partner lab for analysis. Frozen samples must be analyzed within 25 days.