SOP 6 – Chlorophyll *a* Sampling and Analysis

Version 1.01 (June, 2007, 2012)

The following table lists all changes that have been made to this Standard Operating Procedure since the original publication date. Any recommended or required changes added to the log must be complete and concise and promptly brought to the attention of the Project Leader. The Project Leader will review and incorporate all changes, officially complete the revision history log, and change the date and version number on the title page. For complete instructions, please refer to Instructions for Recording Revisions in SOP 13.

Revision History Log:

New Version #	Previous Version #	Revision Date	Author (full name, title, affiliation)	Location in Document and Description of Change	Reason for Change
1.01	1.0	6/1/2012	James M. Caldwell, Water Quality Specialist, USGS Maine Water Science Center	Storing and Shipping: filtered chlorophyll samples must be sent to the laboratory within two weeks of collection	Ensures adequate time for laboratory processing within total allowable storage time

Introduction

This SOP describes the methods for collecting discrete water samples, filtering them through glass-fiber filters, and analyzing them for chlorophyll *a* and other phyto-pigments. Chlorophyll *a* constitutes roughly 1-2% of the dry weight of phytoplankton and consequently is a convenient proxy measure of phytoplankton biomass. For the spatial assessment component of this monitoring protocol, chlorophyll is determined on discrete near-surface samples collected at each of the monitoring stations and analyzed by the methods presented in this SOP. In addition, chlorophyll is monitored continuously during the index period at a single station using a chlorophyll sensor (*in situ* fluorometer) on the YSI 6600EDS sonde. A preliminarily calibration of the chlorophyll sensor is generated using Rhodamine WT dye. This provides approximate chlorophyll values and the ability to evaluate whether the sensor has drifted over time. In order to produce reportable chlorophyll *a* concentrations, however, data from the *in situ* method (using the YSI sensor) must be post-calibrated against discrete samples measured by the extractive method described here. Five ore more discrete water samples are collected for each deployment

of the sonde, and these samples should be dispersed throughout the deployment period. They are collected coincident in time and space with a logging event so that paired samples can be used to generate a correction curve for the YSI chlorophyll sensor.

As designed, the water-quality monitoring protocol requires that 13 stations be visited each week during the four-week index period (six trend stations, six probability stations, and the continuous monitoring station). Several additional samples must be collected at the continuous station, the exact number of which depends on the number of sonde deployments during the index period (see SOP 3 – Continuous Water-Quality Monitoring with the YSI Sonde). Adequate supplies and material must be acquired to accommodate the collection, filtration, and storage of 120 samples per park each season (samples are filtered in duplicate).

There are three basic approaches for the analysis of chlorophyll *a* from phytoplankton extractions. In each case, phytoplankton are collected on a filter and the chlorophyll is extracted in solvent, which is generally 90% acetone. The three methods are distinguished principally by their methods of detection: visible spectrophotometry, fluorometry, or high-performance liquid chromatography (HPLC). HPLC yields the most detailed information about the photosynthetic pigments in the sample, but is relatively expensive and time consuming. Spectrophotometry provides an intermediate level of information on different pigments at an intermediate cost, but requires that large volumes of water be filtered. Fluorometric methods have the advantage of being highly sensitive (requiring less filtering) and relatively inexpensive. An additional benefit is that EPA fluorometric method 445.0 version 1.2 is the one prescribed for use in the National Coastal Assessment (U.S. EPA 2001a, Arar and Collins 1997), and is also the standard method in use by the U.S. Geological Survey National Water Quality Laboratory (with modification, USGS 2005). The fluorometric method has been adopted for NPS NER parks particularly because it will allow for increased comparability between NPS vital signs monitoring and other regional assessments.

In order to achieve data quality objectives as cost effectively as possible (refer to SOP 11 – Quality Assurance and Quality Control), this protocol calls for all chlorophyll analyses to be performed at the same laboratory using the same analytical method. The Atlantic Research Center at CACO has been identified for this task, and will follow the QA/QC requirements of EPA method 445.0 including an initial demonstration of capability and the continued analysis of laboratory reagent blanks, field duplicates and quality control samples as a continuing check on performance. Three parks within the two networks covered by this protocol (ASIS, GATE, and BOHA) have ongoing water-quality monitoring programs. These parks will have the option of either sending all NCBN samples to the Atlantic Research Center at CACO, or continuing to use their existing laboratories and methods while also sending a subset of samples to CACO for laboratory intercomparison purposes. A minimum of 10% of the samples (approximately six per park per year) must be analyzed by both laboratories. These intercomparison samples must be distributed among all days of fieldwork throughout the index period.

Collection and Field Handling of Samples

At each station, a 1 L water sample is collected from a depth of 20-30 cm. It is important that these discrete samples not be biased by pleuston and neuston floating at the water's very surface.

This can be accomplished without the need for special sampling equipment by gathering water directly into the sample container. If there are concerns about potential contact with microbiological or chemical contaminants in the water, any style of sub-surface water grab sampler or a shoulder-length neoprene glove may be used.

Equipment and supplies for sample collection

- 14 1-L amber polyethylene wide mouth sample bottles
- insulated chest large enough to accommodate the above bottles arranged in an upright position
- 10-15 lbs of ice (cubes or crushed)
- optional sub-surface water sampling device

Methods of sample collection

Once on station, select a clean dry sample bottle and label it with the spatial element (i.e. hexagon) number and the time. If sampling at the continuous monitoring station, substitute "continuous station" for the number of the spatial element. Loosen the cap, but hold it in place on the mouth of the bottle. Invert the bottle and lower it to a depth of 20-30 cm. While holding at that depth, tip the bottle upright, allowing air to escape from under the cap. Loosely cap the bottle, bring it to the surface, agitate to rinse, and dump the contents (away from the spot where you are collecting the sample). Repeat this filling procedure a second time, but cap tightly to retaining the contents. Plunge the sample into an ice water bath in the insulated chest (0-4 ˚C). It is imperative that samples be kept cold and dark the entire time they are being held for filtering.

One 1-L grab sample must be collected at each of the spatial survey stations each time they are sampled. If the spatial sampling is conducted as designed, then 6 trend stations and 6 random stations are occupied for each of four weekly survey cruises.

Grab samples must also be collected to calibrate the fluorometer on the YSI sonde at the continuous monitoring station. At a very minimum, a 1-L grab sample shall be collected at the time the sonde is deployed and retrieved, and during each of the four weekly spatial survey cruises. To determine whether additional grab samples are needed review SOP 3 – Continuous Water-Quality Monitoring with the YSI Sonde. For grab samples taken at the continuous logging station, it is very important that the time of collection coincide with a logging event of the continuous sonde. These occur regularly on the quarter hour. It is also imperative that the sample be collected at the same water depth. Any water sampler capable of collecting a 1-L sample from this depth is acceptable. Note that during deployment and retrieval of the sonde, the water sample is collected from a container in which the logging sonde has been submersed, so that the chlorophyll sample and the fluorometer reading are taken from the same water at the same time – see SOP 3 – Continuous Water-Quality Monitoring with the YSI Sonde.

Sample Filtration

Filtering should be performed in subdued light as soon as possible after sampling since algal populations, and thus chlorophyll *a* concentration, can change in a relatively short period of time. The EPA recommended holding time of raw water samples prior to filtration is four hours provided the sample is held at 4 ˚C. This SOP calls for samples to be filtered under controlled laboratory conditions rather than in the field. While this may result in some samples being held for more than four hours, it has several advantages over filtering in the field that make it preferable for Vital Signs monitoring. These include better control over the volume of water being filtered, better control of pressure differential (vacuum) across the filter, ability to work under subdued light, and a shorter station time. This last factor has implications for other Vital Signs such as PAR attenuation that must be measured as close to local apparent noon (for Eastern Daylight Time this is close to 13:00 hrs) as possible.
Minimize the amount of time that raw water samples are held by initiating the filtering procedures as soon as possible after returning from the field, and by filtering samples in the order they were collected.

Equipment, labware, and supplies for sample filtration

- vacuum pump capable of maintaining a vacuum up to 20 KPa (6 in. Hg) and a meter or so of vacuum hose
- filtration apparatus consisting of a 1- or 2-L filtration flask, a 47-mm fritted glass vacuum filter holder with glass filter funnel, and a single-hole rubber stopper to connect the two.
- 47-mm glass fiber filters with a nominal pore size of 0.7 µm, such as Whatman GF/F filters.
- Petri dishes, plastic, 50 X 9-mm
- aluminum foil
- graduated cylinders, 250-mL and 500-mL
- tweezers or flat-tipped forceps
- dry ice or ultra-freezer (-20 ˚C to -70 ˚C)

Filtering methods

All reusable lab ware that comes in contact with chlorophyll solutions should be clean and acid free. An acceptable cleaning procedure is soaking for 4 h in laboratory grade detergent and water, and rinsing with tap water, distilled deionized water and acetone. Assemble the filtration apparatus and attach the vacuum pump via the vacuum hose. Filtering should be performed under very subdued light – the minimum that still allows the technician to perform the task safely.

Each water sample will be filtered onto two duplicate filters. A subset of these will be used for QA/QC purposed by the analytical lab where they are processed. The remainder provide a backup should the first sample analysis be spoiled. Proceed as follows for each water sample:

Place a filter, rough side up (woven side down), on the filter holder and install the filter funnel. It is important that the filter be properly centered on the holder and the funnel be properly attached so that the sample cannot leak around the filter.

Prior to drawing a subsample of water, gently resuspend the particulate material by swirling it three times in each direction.

Pour off a subsample into a graduated cylinder and accurately measure the volume. Pour this subsample into the filter tower of the filtration apparatus and apply a vacuum. Vacuum filtration should not exceed 20 KPa (6 in. Hg). Higher filtration pressures or excessively long filtration times (>10 min) may damage cells and result in loss of chlorophyll. Experience and care will prevent you from attempting to filter excessively large subsamples, which clog the filter. Typically, a sufficient volume has been filtered for fluorometric analysis when a faint color on the filter can just be observed. The fluorometric method is very sensitive and if too much material is captured on the filter, the extraction efficiency will be reduced and chlorophyll will be underestimated.

Do not suck the filter dry with the vacuum. Instead, slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.

Remove the filter from the support/base with tweezers, fold once with the particulate matter inside, lightly blot the filter with a tissue to remove excess moisture, and place it in a Petri dish. On the outside of the Petri dish, record the park code, the sample ID (same as on the water collection bottle), the date, the replicate number (rep1 or rep2), and the volume of water that was filtered. Also record these values on the Chlorophyll Filtering Data Sheet (Appendix E). Wrap the Petri dish with aluminum foil to protect the sample from light, and re-label the outside of the foil identically to the Petri dish itself. Immediately store the sample at -20 ˚C to -70 ˚C in an ultra freezer or on dry ice.

Repeat this procedure for the second replicate then proceed with the remaining water samples. Be certain to check the level on the filter flask regularly and empty as necessary (seawater may damage the vacuum pumps if it overflows the sidearm filter flask). Also be certain to check the vacuum level with each sample.

Storing Samples and Shipping for Analysis

Samples may be stored frozen at -20 ˚C for as long as 3.5 weeks without significant loss of chlorophyll *a* (Arar and Collins 1997, Weber et al 1986). This is the total allowable holding time, so samples should be shipped to the laboratory within two weeks. Prior to shipping, confirm that the receiving laboratory has appropriate sample storage facilities and alert them to expect a shipment requiring immediate attention upon arrival. Express ship all samples on dry

ice using overnight service following applicable federal, state and local shipping regulations for hazardous materials (dry ice). Federal Express will currently ship samples overnight packaged with dry ice at no additional charge over their standard rates. If a Styrofoam insulated box is used, it must be encased in an outer cardboard container. Small insulated coolers (with rigid shell) are also acceptable. All shipments containing dry ice must carry an appropriate hazardous material label (provided by FedEx).

References

Arar, E.J. and G.B. Collins. 1997. U.S. EPA Method 445.0 *In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater phytoplankton by fluorescence. Revision 1.2 .Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S.E.P.A., Cincinnati, Ohio (EPA/600/R-92/121, Nov. 1992). Method and ERRATA SHEET available at <http://www.epa.gov/nerlcwww/ordmeth.htm#marine>

U.S. EPA. 2001. Environmental Monitoring and Assessment Program (EMAP): National Coastal Assessment Quality Assurance Project Plan 2001-2004. United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA/620/R-01/002. 198 pp.

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Weber, C.I., L.A. Fay, G.B. Collins, D.E. Rathke, and J. Tobin. 1986. A review of methods for the analysis of chlorophyll in periphyton and plankton of marine and freshwater systems. Ohio Sea Grant Program, Ohio State University. Grant No.NA84AA-D-00079, 54 pp.