Region 4 U.S. Environmental Protection Agency Laboratory Services and Applied Science Division Athens, Georgia	
Operating Procedure	
Title: Algal Field Sampling	ID: LSASDPROC-516-R2
Issuing Authority: Field Services Branch Supervisor	
Effective Date: April 22, 2023	Next Review Date: March 30, 2026
Method Reference: N/A	SOP Author: Susan Dye

Purpose

This Standard Operating Procedure (SOP) should be followed by LSASD Field Services Branch (FSB) staff when collecting water samples for algal analyses performed by the LSASD Algal Laboratory, including chlorophyll *a*, algal growth potential tests (AGPT), and algal toxins.

Scope/Application

This procedure describes several methods that may be used for the collection of surface water samples for chlorophyll a, AGPT, and algal toxin analyses, depending on water body type and study objectives. This document also describes methods and requirements for sample processing, preservation, storage and transport. On the occasion that field personnel determine any of the procedures described in this section are inappropriate, inadequate or impractical and that another procedure must be used to obtain the desired data, the alternative procedure will be documented in the field logbook, along with a description of the circumstances requiring its use. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

While this SOP may be informative, it is not intended for and may not be directly applicable to operations in other organizations. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

Note: LSASD is currently migrating to a paperless organization. As a result, this SOP will allow for the use of electronic logbooks, checklists, signatures, SOPs, and forms as they are developed, which will also be housed on the Local Area Network (LAN) and traceable to each project.

Table of Contents

1. Introduction

1.1 Summary of Method

The LSASD Algal Laboratory currently analyzes filtered surface water samples for chlorophyll a, conducts the algal growth potential test (AGPT) on whole water samples, and runs tests for the algal toxins microcystin and cylindrospermopsin. Chlorophyll a results provide an estimate of phytoplankton biomass in the water sample, and may be used to assess productivity. The AGPT is a bioassay which measures the maximum growth of a test species of algae in response to bioavailable nutrients in the water sample, as well as additions of nitrogen and phosphorus. Results of the AGPT may be used to assess the trophic status of the water body and to determine which nutrient may be limiting productivity. Algal toxin concentrations are measured using an ELISA (enzyme-linked immunosorbent assay) test and may be compared to recommended advisory levels for drinking water or recreational water.

Surface water samples for these analyses may be collected in the field by dipping the sample container along or below the surface, using a discrete depth sampler, or using a depth-integrated sampling device. Samples for chlorophyll analysis are then concentrated by filtration of a measured volume through a glass fiber filter, which is stored frozen. Samples for AGPT are collected with minimal headspace in the container and stored on ice, or may be collected with headspace sufficient for sample expansion and stored frozen. Samples for algal toxins are collected with headspace to allow for expansion during freezing, which may occur either before or after receipt by the laboratory.

1.2 General Considerations

Samples for algal analyses should be collected according to both water body type and study objectives. Collecting grab samples just below the water surface is usually appropriate for a well-mixed lotic system, which includes most streams, rivers and estuaries. However, in lentic systems such as a lakes and reservoirs, where phytoplankton and nutrients are unevenly distributed throughout the water column, it may be more appropriate to use a depth-integrated sampler in order to collect a representative sample. Discrete depth samples are required when measuring algal biomass at a certain point below the surface, as when collecting samples to calibrate continuous monitoring devices deployed at depth or assessing algal parameters at specific locations in the water column. For algal toxins, samples may be collected along the surface at near-shore locations, in order to target areas of recreational activity relevant to human health. Best professional judgment should be used to determine which sampling method will meet the goals of the project.

When using a depth-integrated sampler, it is usually necessary to conduct a light attenuation profile prior to sampling. Measuring photosynthetically active radiation (PAR) at regular increments from the surface allows determination of the photic zone, the region from the surface to where PAR is 1% of the surface value and begins to constrain photosynthesis. The photic zone typically dictates where phytoplankton occur. Therefore, depth-integrated samples should encompass as much of this region as possible, without

extending below the lower boundary. In the absence of light measurements, a Secchi disk may be used to estimate the photic zone

2 Methodology

2.1 Sample Collection

2.1.1 Equipment and Supplies

- 1 L amber polypropylene bottles* (chlorophyll)
- 2 L wide-mouth Nalgene® bottles (AGPT)
- electrical tape (AGPT, toxins)
- 500 mL amber glass with PFTE-lined cap, or PTGE plastic bottle (toxins)
- sodium thiosulfate (toxins, drinking water only)
- light meter or Secchi disk (optional)
- discrete or integrated depth sampler, or other sampling device (optional)
- large funnel for use with integrated depth sampler (optional)
- 2-4 L mixing container for use with integrated depth sampler (optional)
- cooler with wet ice

*The volume of sample required for chlorophyll filtration will depend on sample turbidity as well as the filter size used. One liter is typically sufficient volume for most sites. However, if sampling very clear water such as a headwater stream or oligotrophic lake, 2 or more liters may be necessary in order to concentrate enough algal cells on the filter. The smaller size filter (25 mm) will require less volume than the larger (47 mm).

2.1.1 Surface Water and Discrete Depth Samples

General procedures for sample collection are described in the Operating Procedure for Surface Water Sampling (LSASDPROC-201). Grab samples are most commonly collected by dipping sample containers below the surface, but may be collected according to any of the other methods, as warranted by field conditions or site accessibility. Discrete depths may be sampled using a Kemmerer or Van Dorn sampler, or using a pump with tubing lowered to the desired depth. However, pumps are less preferable due to the potential for algal cells to be destroyed in the mechanism, and should especially not be used if samples are also being collected for algal taxonomy or algal toxins. Sampling devices and containers should be rinsed with ambient water, away from the designated sampling location, prior to sample collection.

2.1.2 Integrated Depth Composite Samples

Integrated depth samples are recommended for collecting a composite sample representative of the photic zone of lakes and reservoirs, where phytoplankton fluctuate in the water column. A specialized depth-integrated sampler consists of a 2 m section of PVC pipe, with increments marked along the side to measure

depth, a rubber stopper at the top end and a valve at the bottom end. The volume of the depth-integrated sampler is approximately 2 L. Multiple aliquots may be necessary to obtain sufficient volume for all analyses, especially if the photic zone is < 2 m. Since the sampler does not collect a uniform sample, aliquots should be homogenized in the mixing container before pouring into sample containers, unless the entire aliquot will fit in the sample container.

See Section 3 for methods to determine the extent of the photic zone.

- If the photic zone is ≥ 2 meters, collect the sample using the full length of the depth-integrated sampler (2 m). If the photic zone is < 2 meters, sample to the calculated depth using graduated markings on the depth- integrated sampler.
- Rinse sampler in ambient water away from the sampling location.
- Ensure the rubber stopper and bottom valve are both open, then slowly lower the sampler vertically into the water, keeping it as straight as possible.
- When the appropriate depth is reached, replace the stopper firmly, then slowly raise the sampler until the open end is just below the surface.
- Close the valve completely while still below the surface, then pull the sampler out of the water.
    - Note: If any water leaks out due to an incompletely sealed sampler, dump the rest of the sample (away from the sampling location), and start over.
- Position the bottom of the sampler over a funnel placed in the mixing container, release the top stopper, and open the valve.
- Repeat if multiple aliquots are necessary to obtain sufficient volume for all sample containers. Aliquots may be homogenized individually or together, depending on the size of the mixing container.
- Homogenize the composite sample in the mixing container before distributing among sample containers.

2.1.3 Drinking Water Samples

When collecting finished drinking water samples for algal toxins, it is necessary to quench any residual chlorine present by adding sodium thiosulfate. The laboratory will provide sample bottles that contain the appropriate amount of dry sodium thiosulfate for the sampling container. Before collecting the sample, open the tap and allow the system to flush for approximately 5 minutes. Sample bottles should not be rinsed, and care must be taken not to overfill the bottle, which would dilute the reducing agent. At least one duplicate sample per batch of 20 samples should also be collected and analyzed for total residual chlorine.

2.2 Chlorophyll Sample Filtration

2.2.1 Equipment and Supplies

- 25 mm or 47 mm glass fiber filters, nominal pore size 0.7 µm
- plastic or glass filtration assembly to match filter diameter
- plastic or glass filtration flask
- hand-operated or mechanical vacuum pump with tubing
- graduated cylinder
- deionized water
- squirt bottle
- aluminum foil cut into squares (approximately 10 x10 cm)
- laboratory tissues
- gloves
- forceps
- portable freezer or cooler with dry ice (optional)

2.2.1 Filtration Procedure

Samples for chlorophyll analysis should be filtered as soon as possible, with filters frozen immediately after filtration. If necessary, due to sampling logistics or lack of freezer availability in the field, whole water samples may be stored on ice (or refrigerated) in the dark for up to 24 hours prior to filtration.

- Set up the filtration assembly with a glass fiber filter, using gloves and forceps.
- Shake the sample thoroughly and pour a measured aliquot into the graduated cylinder, then transfer to the filter funnel.
- Filter using a vacuum pressure not to exceed 6 inches Hg, and release the vacuum as soon as the sample has passed through the filter, to prevent cell lysis and loss of sample.
- Repeat if necessary until color is visible on the filter. Material should only form a thin layer and not cake up against the funnel sides. Record the total volume filtered.
  Note: If the full measured volume cannot pass through due to a clogged filter, discard the filter, rinse equipment, and start over using a smaller volume of sample.
- Once sufficient volume has been concentrated on the filter, rinse the graduated cylinder with deionized water (DI) and pour the rinsate into the funnel. Filter the rinsate while also rinsing the sides of the filter funnel with DI.
- Remove the filter with forceps, fold in half or quarters to enclose particulate material, blot the outsides gently with laboratory tissue to absorb excess water, then wrap in aluminum foil.
- Place foil packet inside of sample bag labeled with sample information as well as the total volume filtered and freeze immediately.

2.2.2 Quality Control Samples

One field split for every ten samples, and at least one filter blank, should be collected with each sampling event. Field splits may be prepared by homogenizing the sample in the field before pouring into two sample containers or filtering two aliquots out of the same sample container. Filter blanks should be prepared after all samples have been filtered and are obtained by filtering a volume of DI similar to volumes of sample filtered. If filtration of samples occurs over several days, it is recommended that one filter blank be prepared at the end of each day.

2.3 Sample Storage

Whole water samples for chlorophyll analysis may be kept on ice in the dark for up to 24 hours prior to filtration but should be filtered as soon as possible and then immediately frozen. Chlorophyll filters can be stored frozen at −20 ˚C (see Section 4.4) for as long as 24 days without significant loss of chlorophyll a. Samples for AGPT should be collected with minimal headspace and stored in the dark on ice or in the refrigerator at 4 °C (see Section 4.4). Samples may also be frozen if the laboratory will not receive them within 5 days of collection, or to facilitate storage and/or transport. If samples will be frozen, headspace of approximately 1-2” should be left in the container to allow expansion of ice. Electrical tape (or similar) should be used around the cap to prevent sample loss or contamination. AGPT samples can be stored at 4 °C for up to 5 days prior to sample processing or may be frozen at −20 ˚C for up to 180 days.

Samples for algal toxins should be stored on ice or in the refrigerator at 4 °C (see Section 4.4). Samples may also be frozen if the laboratory will not receive them within 48 hours of collection, or to facilitate storage and/or transport. Since all samples will eventually be frozen, either in the field or after receipt by the laboratory, allow sufficient headspace to accommodate expansion. Glass bottles may be placed on their side to reduce the chance of breakage. Electrical tape may be used around the caps to prevent sample loss or contamination. The holding time for toxin analysis is 14 days, stored at 4 °C for up to 48 hours and then frozen at −20 ˚C.

2.4 Sample Transport

Once frozen, chlorophyll filters must stay frozen. A freeze-thaw cycle can cause algal cells to lyse, which releases the chlorophyll molecule and accelerates the degradation process. Therefore, transport of filtered samples from field sites to the sample custody room requires either a portable freezer or a cooler with dry ice. If using a portable freezer, the temperature should be recorded in the project logbook when samples are added or removed, as well as each day of storage. Time periods with disruption of power to the freezer (e.g., moving the freezer from hotel room to vehicle) should be minimized. Keeping ice packs or frozen water samples in the portable freezer can help to maintain temperature during these periods, in the absence of a power supply.

If using dry ice, at least 10 pounds of ice are necessary per day, and should be placed in a cooler just large enough to fit contents. Samples should be maintained as close to the

block of dry ice as possible, with any empty space in the cooler filled with packing material. The cooler should be sealed tightly, but have a small vent hole for release of carbon dioxide gas as the ice sublimates. If shipping is necessary, chlorophyll sample filters should be shipped overnight on dry ice, following appropriate shipping regulations.

AGPT samples may be stored on ice or frozen, as described in Section 2.3. If freezing both chlorophyll and AGPT samples for a project, freezer temperature should be monitored when adding chlorophyll filters, since AGPT samples take hours to freeze and may affect the freezer temperature during the process. If shipping is necessary, AGPT samples may be shipped overnight in a cooler of wet ice, regardless of whether samples were frozen prior to shipment.

Algal toxin samples may be stored and transported on wet ice up to 48 hours from collection but may also be frozen and shipped on dry ice, as described for filtered chlorophyll samples above.

3 Determining the Photic Zone

When collecting an integrated depth sample, the goal is to obtain a composite sample representative of the entire photic zone. The lower limit of the photic zone is the depth at which photosynthetically active radiation (PAR) is 1% of the surface value. This depth can be determined using a submersible light meter. If a light meter is not available, the photic zone may be estimated using a Secchi disk.

3.1 Light Meter

Ensure that the light meter is attached to a cord marked at regular intervals, measured from the surface of the light sensor. The unit should be set to measure in photosynthetic photon flux density, usually expressed in µmol photons m^{-2} s^{-1}. Conduct measurements on the side of the boat or sampling platform with consistent light availability.

- Take one measurement just below the water surface.
- Lower the meter slowly through the water column, taking measurements at regular intervals (typically 0.5 m or 1 ft increments).
- Continue until readings decline to approximately 1% of the subsurface value, which marks the lower boundary of the photic zone. More frequent readings may be necessary in this region in order to identify the target depth.
    - **Note: On partly cloudy days, this process may need to be repeated if light availability changes while obtaining measurements.**

3.2 Secchi Disk

Ensure that the Secchi disk is attached securely to a measuring tape or rope that has been calibrated to the surface of the disk. Remove sunglasses and hat to reduce visual bias, and take measurements on the shady side of the boat or sampling platform.

- Slowly lower the disk until it is no longer visible, record that depth, then slowly raise the disk and record the depth at which it reappears.

- Average the disappearance and reappearance values to obtain the Secchi depth, then multiply this by 2 to obtain the approximate depth of the photic zone.

4 Definitions

4.1 Phytoplankton

Free-floating microscopic photosynthetic organisms, including algae and cyanobacteria, present in the water column of an aquatic ecosystem.

4.2 Trophic Status

A classification system, especially for lakes, in which the productivity of the ecosystem is ranked according to low (oligotrophic), moderate (mesotrophic), high (eutrophic) and extremely high (hypereutrophic) nutrients and plant growth.

4.3 Storage Temperatures

4° C means storage at a temperature between 0 °C and 6 °C.
−20 °C means storage at a temperature < −15 °C.

References

American Public Health Association (APHA), American Waterworks Association (AWWA), and the Water Environment Federation (WEF). 2017. “Standard Methods for the Examination of Water and Wastewater.” 23rd Edition. Washington, D.C.

LSASD Operating Procedure for Algal Growth Potential Testing, ASBPROC-700, Most Recent Version.

LSASD Operating Procedure for Determination of Chlorophyll a by Fluorescence, Modified Method, LSASDPROC-702, Most Recent Version.

LSASD Operating Procedure for Determination of Chlorophylls by Spectrophotometry, LSASDPROC-703, Most Recent Version.

LSASD Operating Procedure for Surface Water Sampling, LSASDPROC-201, Most Recent Version.

LSASD Operating Procedure for Total Cylindrospermopsin by ELISA, LSASDPROC-716, Most Recent Version

LSASD Operating Procedure for Total Microcystins and Nodularins by ELISA, LSASDPROC-716, Most Recent Version

Revision History

History	Effective Date
Replaced Chief with Supervisor; General formatting revisions.	April 22, 2023
LSASDPROC-516-R1, Algal Field Sampling, replaces SESDPROC-715-R0 General: Edited to reflect LSASD name change, updated references, applied new SOP formatting, and corrected typographical errors. Changed procedure number code to 500 range for WQS procedures. Changed from 715 to 516. Sections 1.1, 1.2, 1.2.1.1, 2.3, 2.4: Added information about algal toxin analyses. Section 2.1.4: Added new section about collecting drinking water samples for algal toxins. Section 4: Removed the definition of oligotrophic, which is included in the definition of trophic status.	March 30, 2022
SESDPROC-715-R0, Algal Field Sampling, Original Issue	March 22, 2018