

Chlorophyll-*a* Sample Collection and Processing

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Preface

The Watershed Protection Branch (WPB) of the Georgia Environmental Protection Division (GAEPD) has created a series of Standard Operating Procedures (SOP) establishing uniform methods for the field collection of data, document control, quality assurance, laboratory safety, as well as other activities. These guidance documents were developed to document, and ensure, the validity of measurements, analyses, and the representativeness of samples collected. This is necessary in the event of a dispute with other parties regarding data collection techniques and the resulting quality of field information. Enforcement activities by the Branch require full documentation on particulars of data collection and the equipment used to collect it. All Branch Associates who collect samples or field data must be familiar with the measures outlined in the appropriate SOPs.

Requirements pertaining to specifics of sample collection for certain parameters are specified in federal regulations under the authority of the Clean Water Act (CWA) and the National Pollutant Discharge Elimination System (NPDES) permitting program. The most widely applicable guidance at this level is *Title 40 of the Code of Federal Regulations (40CFR)*. The procedures and techniques given in *40CFR* are updated periodically by the United States Environmental Protection Agency (U.S. EPA) and field workers are advised to consult the latest revision for proper procedures and new developments. In addition, the SOPs utilized by the Branch should be reviewed annually to certify their concurrence with federal statutes. Other references used in developing each SOP are cited at the conclusion of the individual documents.

The collection protocols in *40CFR* are in many instances based on the concern for quality assurance. As such, each SOP will contain a section devoted to maintaining and improving the quality of data collected. ‘Quality Assurance and Quality Control’ sections contained within individual SOPs are not meant to replace the overall Quality Assurance Project Plan document prepared for the Branch, but rather, are provided as supplemental data for each specific, standardized activity.

This document is dynamic and will be continually revised as new developments warrant. As the Branch assumes more responsibilities for studying and sampling in new investigational areas, it is anticipated that additional SOPs will be required.

A. Introduction

The WPB of the GAEPD is responsible for managing the surface waters of the State of Georgia. The WPB works to ensure that Georgia's surface waters are of a quality and quantity sufficient for fulfilling multiple uses within the state by controlling nonpoint sources of pollution, managing storm water discharges, and regulating the amount of discharges to, and withdrawals from, surface waters. These tasks are accomplished through the issuance of NPDES permits to local governments and industry for the discharge of treated wastewater and to local governments, industry, farmers and subdivisions for surface water withdrawals. However, none of these tasks would be possible without the vital data collected through water quality monitoring.

Water quality monitoring is integral to the WPB's successful management of the waters of the State. Monitoring and studies conducted by the WPB can be broadly categorized as either enforcement or non-enforcement related activities. The enforcement related monitoring includes water enforcement case investigations, NPDES compliance sampling inspections (CSIs), some diagnostic evaluations of municipal and industrial wastewater treatment plant discharges and monitoring of sewage spills into surface waters. Monitoring conducted that does not have a specific enforcement objective includes trend monitoring, surveys to verify issued permit limits, waste load allocation and model calibration studies, and other intensive surveys for documenting water quality.

Monitoring is accomplished through surface water sampling events planned in accordance with the type, amount, and time frame of data required. Surface water sampling techniques and equipment have been designed not only to minimize possible contamination of the chemical and physical integrity of the sample, but also to provide a sample that is representative of the waterbody under investigation. If the guidance provided in this SOP is followed, an unbiased, representative sample of the surface water should be obtained.

- | Note: Because studies and data derived from non-enforcement type investigations could be used for enforcement purposes at a later time, both investigations follow the procedural guidelines presented in this document.

B. Purpose and Applicability

The purpose of this SOP is to establish uniform procedures for sampling chlorophyll-*a* from lakes, reservoirs, and estuaries in the State of Georgia. Chlorophyll-*a* is used as an indicator of algal biomass and trophic status of lentic systems. The procedures outlined in this SOP are applicable to all Branch Associates who collect or assist in the collection of chlorophyll-*a* samples in support of water quality and compliance monitoring.

C. Summary of Method

Chlorophyll-*a* samples are collected monthly from lakes, reservoirs, and estuaries during the growing season (April-October) when primary productivity is highest. Samples are collected at one (1) meter intervals within the photic zone and homogenized, generating a single photic zone composite sample for analysis.

Composite samples are transferred and stored in a brown, Nalgene container on ice until further processing. A known volume of water (i.e. 250 mL) is then filtered from each site, along with a beginning and ending blank.

Filters are wrapped in aluminum foil, placed in light-proof brown plastic bags, and flash frozen with dry ice or placed in a cooler on ice until they can be transferred to a freezer. Sample

identification labels, detailing the collection date, collection time, and responsible Associate(s) for collecting the sample are adhered to the sample containers.

Chain of Custody forms (Orange Sheets) are completed, the samples are packed in dry ice and shipped to an appropriate State certified laboratory.

D. Definitions

1. **Clean Water Act (CWA)** – As amended in 1977, the CWA established the basic structure for regulating discharges of pollutants into the waters of the United States. It gave the U.S. EPA the authority to implement pollution control programs such as setting wastewater standards for industry. The CWA also contains requirements to set water quality standards for all contaminants in surface waters. The CWA made it unlawful for any person to discharge any pollutant from a point source into navigable waters, unless an NPDES permit was obtained under its provisions. It also funded the construction of sewage treatment plants under the construction grants program and recognizes the need for planning to address the critical problems posed by nonpoint source pollution.
2. **Compliance Sampling Inspections (CSI)** – Studies that involve splitting effluent samples with the permittee to monitor permitted discharges for compliance with NPDES permits.
3. **Depth Composite Sample** – A sample taken over multiple depths where equal water aliquots are collected and combined into one, homogenous sample.
4. **Discrete Depth Sample** – Water collected from a specified depth in the water column using a specialized sampling device.
5. **Grab Sample** – An instantaneous sample from one point in the waterbody. This produces a sample that is representative of the surface water's quality at the moment the sample was taken.
6. **Intensive Survey** – A study that incorporates many different fields of research to fully understand the complexity of a water system. In most cases, this includes tributary and lake sampling for water quality characteristics, biotic life, sediment quality, and flow status. These studies tend to be anywhere from a few days to a year in duration.
7. **Multiparameter Water Quality Probe (Sonde)** – A water quality meter consisting of multiple probes for analyzing parameters of interest. For the purposes of GAEPD's monitoring, these probes generally consist of pH, dissolved oxygen (DO), conductivity, and temperature.
8. **National Pollutant Discharge Elimination System (NPDES)** – As authorized by the CWA, the NPDES permit program controls water pollution by regulating point sources that discharge pollutants into waters of the United States. Point sources are discrete

conveyances such as pipes or man-made ditches. Individual homes that are connected to a municipal system, use a septic system, or do not have a surface discharge do not need an NPDES permit; however, industrial, municipal and other facilities must obtain permits if their discharges go directly to surface waters.

9. **Special Response Investigation** – A study conducted in response to a complaint or request submitted by a member of the general public, a water treatment facility operator, a member of a municipal government, a citizen’s action group, etc.
10. **Surface water(s) of the State or surface water(s)** – Any and all rivers, streams, creeks, branches, lakes, reservoirs, ponds, drainage systems, springs producing in excess of 100,000 gallons per day, and all other bodies of surface water, natural or artificial, lying within or forming a part of the boundaries of the State that are not entirely confined and retained completely upon the property of a single individual, partnership, or corporation.
11. **Vacuum Pump** – Commercially available pump that uses flexible pumping to create small amounts of negative pressure to a filtering apparatus. Some models are battery operated, but manual models are also available. Both are suitable for the filtering of sample water.
12. **Van Dorn sampler** – A device for obtaining water samples at a discrete depth. It is a plastic tube with a 10-cm inner diameter, open to the water at both ends. Each end is equipped with a cap and connected by an elastic band. The sampler is lowered to a specified depth, and a weighted messenger is used to trip both caps shut, which seals the tube.

E. Health and Safety Warnings

Collection and analysis of surface water samples can involve significant risks to personal health and safety. The sampler should treat all water samples as if they contain a chemical contaminant or biological agent that could cause illness and should minimize exposure to both the sample and sampling medium. The sampler should wear appropriate personal protective equipment (PPE) and appropriate clothing when conducting sampling events. Planning for any type of field sampling should include extensive health and safety considerations including required training (CPR, First Aid, Boating Safety), PPE, and degree of personal, physical condition in accordance with Federal, State, or organizational requirements. Boating safety equipment should be readily available on all boats including: extra keys, flares, throw cushion, horn, personal flotation devices (PFDs), paddles, flashlight, toolbox, and boat notebook with service/fuel record.

F. Cautions

All Associates conducting sampling from boats should wear PFDs. During extremely hot weather, be sure to pack plenty of fluids and drink often to ward off the risk of heat exhaustion and heat stroke. Associates should also wear sunscreen, especially on sunny days. Field sampling should NEVER be conducted alone. Sampling teams should always consist of a minimum of two

Associates.

G. Interferences

The purpose of representative sampling is to characterize the true picture of the surface water at the time of sampling. Contaminants introduced into the sample containers through careless handling, or by using “dirty” preservatives can bias the true picture. Common contaminants include, but are not limited to:

- Careless handling of sample container lids
- Use of containers that have been lying unprotected in work vehicle for extended periods of time
- Use of previously used sample containers without proper cleaning and rinsing
- Careless transfer of sample from one container to another
- Failure to pre-rinse sample compositor with sample water between sites

H. Personnel Qualifications

All Branch Associates who collect surface water samples or field data must be familiar with the procedures outlined in this document. In all aspects of water quality planning and field assessment activities, safety is to be addressed and treated as a critical element. The Georgia DNR *Safety Manual* is to be consulted, and its policies, protocols, and procedures are to be incorporated and implemented in WPB field activities.

I. Procedural Steps

Equipment and Supplies

- Vehicle capable of carrying or towing boat
- Boat that meets the size requirements of the lake/estuary, equipment, and/or Staff
- Boat safety equipment including:
 - Extra keys
 - Flares
 - Throw cushion
 - Horn/Whistle PFDs
 - Paddles
 - Flashlight
 - Toolbox
 - Boat notebook with service/fuel record.
- Field Forms/Notebook,
- Container labels,
- Writing instruments
- Carboy for mixing integrated sample
- Container with supply of deionized (DI) water
- Meter stick
- Photometer (e.g. Li-Cor)

- Secchi Disk
- Water collection device (e.g., Van Dorn)
- Rope for Van Dorn marked off in meters
- Brown opaque containers
- Chlorophyll-*a* filtration kit that includes:
 - Pressure/vacuum pump with battery or hand-pump
 - Graduated cylinder (250-500 mL size)
 - Filtering flask (1000 mL or larger)
 - Tubing connecting pump to flask, stainless steel vacuum filter holder
 - 47 mm Whatman glass chlorophyll-*a* filters or like product
 - Aluminum foil
 - Forceps (at least 2)
 - 1" X 2" filter storage zip-lock bags for individual filters,
 - 2" X 3" zip-lock opaque brown bags for pairs of similar filters
 - 6" X 8" (or similar) clear zip locks bags for storage of all brown bags from a day's collection
 - Labels for zip-lock bags containing individual filters
- Cooler(s) with wet ice
- Cooler(s) with dry ice
- Chain of Custody form (Orange Sheet) to be filled out for each batch of delivered chlorophyll-*a*.

Procedure

- Load boat with all listed gear except for chlorophyll-*a* kit and dry ice cooler.
- Prior to departing in a boat, all Associates should have on high-visibility U.S. Coast Guard approved personal flotation devices. Once the boat is launched, proceed to the sampling location, and anchor at the sample site.

1. Photic Zone Measurement using the Li-Cor

Most chlorophyll-*a* is analyzed from a depth composite water sample that encompasses the entire photic zone. The depth of the photic zone is measured using a photometer. If a photometer is not available use the Secchi disk.

- At the site, remove the photometer (e.g. Li-Cor) from the storage case. There are two sensors, one is the surface sensor and the other is the underwater sensor that is attached to a metal weight. The surface sensor should be plugged into I1 and the underwater sensor should be plugged into I2 on the data logger. .. Place both sensors on a flat surface at the same level. Make sure not to place the surface sensor in a shaded area. Turn the data logger on and toggle between I1 and I2 to record readings of each sensor.
- Toggle the unit to M1. Lower the underwater sensor into the water on the sunny side of the boat until the data logger reads 100. At that point, you have reached where

1/100 of the amount of surface light can penetrate and this is the edge of the photic zone. Measure and record this depth (e.g. 6.23 m).

2. Estimating Photic Zone using Secchi Disk

If a photometer is not available, a Secchi disk reading can be used to estimate photic zone. The Secchi disk depth is a measure of water clarity and a basic water quality diagnostic tool. It should be noted that using a Secchi disk is very subjective, and different Associates may get very different results. For this reason, it is not recommended that a Secchi disk be used for determination of photic zone on a regular basis.

- Remove any visual aids with polarized lenses before performing Secchi disk readings.
- The Secchi disk is lowered slowly on the shaded side of the boat until the disk is not visible.
- Raise the disk slowly until it is barely visible. Lower and raise the disk again. Lower the disk a third time until it is just beyond visibility. This is the Secchi disk depth and is measured from the water surface to the disk. Measure and record this depth. Now that the Secchi disk depth has been determined, multiply that depth by three (3). That calculated depth is the estimate of the photic zone depth.

3. Collect a Photic Zone Composite Sample

Once the photic zone has been determined, discrete water samples will be taken at the surface, 0.1 meter (m) depth, and at 1 m intervals throughout the photic zone. The Van Dorn sampling device volume is about 2 liter (L), and the carboy capacity is about 20L. If the photic zone is greater than 7 m, adjust water volume from each grab accordingly as it is poured into carboy to ensure the total volume for composite sample does not exceed 20L.

- Determine the number of discrete water samples to be collected. The depth of the photic zone is be rounded to the nearest whole meter depth. If the photic zone is 6.23 meter (m) round the depth to 6 m. If the photic zone is 6.84 m round the depth to 7 m.
- Open both caps on either end of the water sample collecting device or Van Dorn and secure them in the open position.
- Lower the Van Dorn to 0.1 m into the water column, drop the weighted messenger to trigger both caps to shut, and collect a sample.
- Raise the Van Dorn to the surface and carefully open one cap of the Van Dorn and pour into composite carboy.
- Repeat discrete water sample collection at one (1) meter intervals by lowering the Van Dorn one meter at a time until you reach the bottom of the photic zone and have collected a sample from each meter throughout the photic zone until the edge of the photic zone is reached.
- Once the composite sample has been completed, homogenize samples in a carboy to

create a single depth-integrated composite. Gently rock or invert the carboy to assure even mixing.

- Rinse the designated chlorophyll-*a* container with the composite sample before filling.
- Decant at least 1000 mL into 1200 mL opaque brown storage container. The container should be filled only to the shoulder to allow space for mixing the sample before filtering.
- Record the site name, Mon Loc ID, time, date and collector on the container label and place the brown container on wet ice.
- Repeat this process at each site.

4. Filter the Photic Zone Composite Chlorophyll-*a* Sample

Upon the return to dry land at the end of the day, set up for chlorophyll-*a* filtration as soon as possible. Work out of direct sun and wind, if possible (sheltered picnic facilities are an excellent choice).

- Fill squirt bottle with DI water.
- Rinse graduated cylinder and filtering apparatus with DI water before initial use.
- Assemble flask and filtering apparatus including a new 47 mm filter.
- Measure 250 mL of DI water in the graduated cylinder and pour into funnel. This is the *Beginning Blank*. Use the vacuum or hand pump to create suction in the flask to pull the water sample through the filter. Create no more than 3 psi of pressure.
- After filtration is complete, remove funnel and use forceps to fold 47 mm round filter in half. Fold it in half again (effectively creating a pie shape one quarter the size of the original filter).
- Using the forceps, place the filter in the center of a 2" X 2" piece of aluminum foil and fold in such a way that the filter is completely covered and protected from light.
- Place the folded aluminum in a 1" X 2" zip lock and label as "Beginning Blank" with the date, filtered time, and number of sample water milliliters filtered through the filter.
- Place this bag into the 2" X 3" brown plastic bag, and store in a 6" X 8" bag on dry ice or in freezer
- Pick one of the brown containers from the cooler containing a collected integrated sample. Gently invert the container a few times.
- After placing a new filter on the screen, attach funnel again to flask.
- Measure 250 mL of water into the graduated cylinder and pour into the funnel. Filter sample as described above. (250 mL may be too much sample depending on site)
- After filtering is complete, remove the filter, fold it into quarters, wrap it in aluminum foil, place in a 1" X 2" zip lock, making sure to properly label with site name, Mon Loc ID, volume filtered, date, and time sample was collected in the field. Place this bag into the 2" X 3" brown plastic bag. This is the first replicate of sample #1.
- Rinse the graduated cylinder, flask, and filtering apparatus between filtering. Place a new filter on the screen.
- Gently invert the same brown sample container again and decant the same volume filtered for the first replicate into the graduated cylinder.

- Repeat the process outlined above. This is replicate #2 for the site.
- Rinse the graduated cylinder and funnel with DI water from the squirt bottle between sites to prevent cross contamination.
- Remember to empty the flask about every 5th filtration, depending on the volume of the specific flask.
- Repeat this process for each water sample, filtering 2 replicates for each site.
- After the final replicate, rinse the funnel and graduated cylinder one last time, then filter one last 250 mL volume of DI water through a new filter. Process in the same fashion as outlined above. This becomes the final filtration. Label this as the “*Ending Blank*”.
- Place the first replicate from each site along with the *Beginning* and *Ending Blank* into one 6”X8” bag. Place the second replicates in a separate 6”X8” bag for backup purposes.
- Store all samples on dry ice or in a freezer until delivered to the proper lab for processing.
- Disassemble filter apparatus. Remember to empty the squirt bottle and secure all supplies so the kit is ready to be used for the next sample run. Complete lab Chain of Custody forms (Orange Sheets) for the filters, making sure to include filtration volume, and deliver to the appropriate lab. Holding time for chlorophyll-*a* filters is 22 days. It is best to send the filtered samples to the lab within seven days of collection..
- Store all equipment and supplies in a safe and dry environment where they will be ready for use on the next field trip.

J. Data and Records Management

Complete a lab Chain of Custody form (Orange Sheet) for the batch of filters. This document can be custom designed, but must include information that details the project name, sample collector, date collected, station number, and station name for each site, time the sample was collected, amount filtered, time filtered, and time frozen for each sample and include space for the lab to attach a printed label. See current chlorophyll-*a* lab documents for example. Make a copy of the document for your records once receiving personnel have marked it with the time and date.

Data and records will be managed according to the policies outlined in the GAEPD SOP# EPD-WPMP-1. Any deviation from the policies outlined in the SOP should have prior approval from first the Unit manager, and then the Program manager, and be documented accordingly.

K. Quality Assurance and Quality Control

1. Field Blanks

For each day of sampling, beginning and ending blanks are filtered and processed identically to samples. This is to determine if there has been any cross contamination in processing chlorophyll-*a* samples.

2. Field Replicates

- In accordance to the Georgia Quality Assurance Project Plan (QAPP) section B5.2 (located at <https://epd.georgia.gov/watershed-protection-branch/watershed-planning-and-monitoring-program/monitoring#toc-sops-and-qapp>), Georgia EPD associates will collect replicate samples at 10% of all sample events (this is subject to change based on the project plan and/or laboratory constraints).
- For each day of sampling that requires a replicate QC sample, the site to receive the replicate QC sample should be determined prior to going out in the field.
- Replicates must be obtained by repeating the entire sampling procedure after the initial sample event is processed; that is, the van Dorn and carboy must be rinsed, and the samples collected and filtered in the same manner as the initial sample.

3. Sample Handling

After collection, all sample handling should be minimized. Investigators should take extreme care to ensure that samples are not contaminated. If samples are placed in an ice chest, investigators should ensure that melted ice cannot cause the sample containers to become submerged, as this may result in sample cross-contamination. Plastic bags, such as Zip-Lock® bags or similar plastic bags should be used when small sample containers (e.g., VOC vials or bacterial samples) are placed in ice chests to prevent cross-contamination.

Additional quality assurance and quality control information regarding surface water monitoring may be found in the GAEPD's Quality Assurance Program Plan (2008-12) available online at www.gaepd.org.

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