Chlorophyll-*a* Sample Collection and Processing

January 2008

Prepared by: Chip Cutcliff **Date: 2/21/08**

Title: Environmental Specialist

Reviewed by: Jeremy Smith **Date: 2/22/08**

Title: Environmental Program Manager I

Approved by: Dr. Elizabeth Booth **Date: 2/27/08**

Title: Environmental Program Manager II

Annual Reviewer				
Date				

Table of Contents

Preface…… 3

A. Introduction…… 3

B. Purpose and Applicability…… 4

C. Summary of Method…… 4

D. Definitions…… 5

E. Health and Safety Warnings…… 6

F. Cautions…… 6

G. Interferences…… 7

H. Personnel Qualifications…… 7

I. Procedural Steps…… 7
1. Photic zone composite sampling…… 7

J. Data and Records Management…… 11

K. Quality Assurance and Quality Control…… 11

L. References…… 11

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 SOP's.

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 (40 CFR)*. The procedures and techniques given in *40 CFR* are updated periodically by the United States Environmental Protection Agency 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 *40 CFR* 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 documents 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 Watershed Protection Branch (WPB) of the Georgia Environmental Protection Division (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 National Pollutant Discharge Elimination System (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 water body under investigation. If the guidance provided in this standard operating procedure (SOP) is followed, an unbiased, representative sample of the surface water should be obtained.

> *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 and reservoirs in the State of Georgia. Chlorophyll-*a* is used as an indicator of alagal 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 either on a quarterly or monthly basis. Lakes that have established chlorophyll-*a* standards are sampled once per month during the growing season (April-October) each year when primary productivity is highest. Lakes within the rotating river basin schedule are sampled once per quarter that calendar year. Samples are collected at one (1) meter intervals at with in the photic zone and homogenized, generating a single photic zone composite sample for analysis. Composite samples are stored in a brown, Nalgene bottle 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 and placed in light-proof, brown bags and are

then flash frozen with dry ice. Sample identification labels, detailing the collection date, collection time, and responsible Associates are adhered to the sample bottles. Laboratory Source Documents (Green Sheets) are completed, the samples are packed in ice, and then shipped to an appropriate, State certified laboratory.

D. Definitions

1. **Van Dorn sampler** – is a device for obtaining samples of water at a discrete depth. It is a plastic tube with a 10 cm inner diameter, and open to the water at both ends. Each end is equipped with a cap and connected by an elastic rope. The sampler is lowered to a specified depth, and a weighted messenger is used to trip both caps shut and seal the tube.

2. **Clean Water Act (CWA)** – As amended in 1977, the Act 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 Clean Water Act also continued requirements to set water quality standards for all contaminants in surface waters. The Act made it unlawful for any person to discharge any pollutant from a point source into navigable waters, unless a permit was obtained under its provisions. It also funded the construction of sewage treatment plants under the construction grants program and recognized the need for planning to address the critical problems posed by nonpoint source pollution.

3. **Compliance Sampling Inspections (CSI)** – Studies which monitor permitted discharges for compliance with NPDES permits.

4. **Composite Sample** – In this case, a sample that is taken over multiple depths. Equal aliquots water are collected and combined into one, homogenous sample.

5. **Discrete Depth Sample** – A discrete depth sample collects water from a specified depth in the water column using a specialized sampling device.

6. **Grab Sample** – A grab sample is an instantaneous sample from one point in the water body. This produces a sample that is representative of the surface water's quality at the moment the sample was taken.

7. **Intensive Survey** – An intensive survey is 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 a minimum of a year in duration.

8. **Multi-probe (Data sonde) –** A water quality meter consisting of multiple probes for analyzing parameters of interest. For the purposes of GAEPD's monitoring, these probes will generally consist of ph, dissolved oxygen (DO), conductivity, and temperature.

9. **National Pollutant Discharge Elimination System (NPDES)** – As authorized by the Clean Water Act, the National Pollutant Discharge Elimination System 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.

10. **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.

11. **Special Response Investigation** – A special response investigation is 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.

12. **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 which are not entirely confined and retained completely upon the property of a single individual, partnership, or corporation.

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 minimize exposure to both the sample and sampling medium. The sampler should wear appropriate personal protective equipment 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), personal protective equipment, and degree of personal, physical condition in accordance with Federal, State, or organizational requirements. Boat safety equipment should readily available on all boats including: extra keys, flares, throw cushion, horn, life jackets, paddles, flashlight, toolbox, and boat notebook with service/fuel record.

F. Cautions

All Associates conducting sampling from boats should wear personal flotation devices.

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 bottles which 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

1. Photic zone composite from boat – Most chlorophyll-a is analyzed from a composite water sample that encompasses the entire photic zone. The depth of the photic zone is measured using a photometer. Once the photic zone has been determined, samples are taken at one (1) meter intervals until the edge of the photic zone is reached. Samples are then homogenized to create a depth-integrated composite.

<u>Equipment and Supplies</u>
Vehicle capable of carrying or towing boat
Boat that meets the size requirements of the lake, equipment, and/or staff
Boat safety equipment, including extra keys, flares, throw cushion, horn, life jackets, paddles, flashlight, toolbox, and boat notebook with service/fuel record.
Field Notebook, writing instruments
Carboy for mixing integrated sample
Container with supply of deionized (DI) water
Meter stick
Underwater photometer to establish the photic zone
Secchi Disk

Water collection device (e.g.Van Dorn)
Rope for Van Dorn marked off in half meters
Brown opaque bottles
Chlorophyll filtration kit, which 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, supply of 47 mm Whatman glass chlorophyll filters or like product, aluminum foil, at least 2 forceps, 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 days collection.

Cooler(s) with wet ice
Cooler(s) with dry ice
Chain of custody form (green sheet) to be filled out for each batch of delivered chlorophyll**.**

Procedure

1. Boat is loaded with all listed gear except for chlorophyll kit, DI water, and dry ice cooler.
2. Prior to departing in a boat, all staff 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.
3. At the site, remove the photometer (e.g. Li_Cor) from the storage case. There are two senors, one for the surface and an underwater sensor, which is attached to a metal weight. **(**If a photometer is not available use Secchi disk to estimate photic zone. See below.)**
4. The surface sensor should be plugged into I1 and the underwater sensor into I2 on the data logger. Turn the data logger on and set it to I2.
5. Lower the underwater sensor into the water until the data logger reads 100. At that point, you have reached where 1/100 of the amount of surface light can penetrate and the edge of you photic zone. Measure and record this depth (e.g. 6.23 m).
6. Lower your sample-collecting device (Van Dorn) one meter into the water column and collect a sample. Pour into composite carboy.
7. Repeat this process, lowering one meter at a time until you reach the bottom of the photic zone and have collected a sample from each meter throughout it. (For example, if the photic zone depth is 6.23, samples should be taken at depths 1-6 m. If the edge of the photic zone is at 6.63, samples should be taken at 1-7 m.). The Van Dorn sampling device volume is about 2L, and the carboy holding capacity is usually 20L. If the photic zone is greater than 10 meters, adjust water volume from each grab accordingly as it is deposited into carboy to guarantee that total volume for sample does not exceed 20L.
8. Once the composite sample has been completed, shake vigorously to assure even mixing.

9 Decant at least 1000 mL into a numbered, 1200 mL opaque brown storage bottle, record the place, time and number of the bottle in the field book and place the brown bottle on wet ice.

10 Repeat this process at each site. Upon return to dry land at end of day, set up for chlorophyll *a* filtration as soon as possible.

11 Work out of direct sun and wind if possible (sheltered picnic facilities are an excellent choice).

12 Assemble flask and filtering apparatus. Fill squirt bottle with DI water. Rinse graduated cylinder and filtering apparatus with DI water before initial use.

13 Measure out 250 mL of DI water in the graduated cylinder, and pour into funnel. This is your *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 7 psi of pressure.

14 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).

15 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.

16 Place the folded aluminum in a 1" X 2" zip lock and label with the date, location, station number, sample number, number of sample water millimeters filtered thru the filter, replicate number, and collectors name. This becomes you *beginning blank*.

17 Put this bag into the 6" X 8" bag, which has already been marked as to which lake(s) samples it is to hold, and place on dry ice.

18 Pick one of your brown bottles from the cooler containing a collected integrated sample. Shake vigorously.

19 After placing a new filter on the screen, attach funnel again to flask.

20 Measure 250 mL of water into the graduated cylinder and pour into the funnel. Filter sample as stated in step #13. After filtering is complete, repeat steps #14-16. This becomes your first replicate of sample #1.

21 Shake the same brown sample bottle again and decant a second 250 mL into the graduated cylinder. Repeat the process. This becomes replicate #2 for the site.

22 Take the two properly notated 1" X 2" bags containing replicates one and two from the first sample and place them into one of the dark brown 2" X 3" bags. Place this brown bag in the larger zip lock with the beginning blank and store on dry ice. You have completed processing the first sample.

23 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 5^{th} filtration, depending on the volume of your specific flask.

24 Repeat this process for each water sample until you have filtered 2 replicates for each site.

25 After the final replicate, rinse the funnel and cylinder one last time, then filter one last 250 mL volume of DI water through a new filter. Process in the same

fashion as the beginning blank from steps 13-17. This becomes your final filtration of the batch.

26 Disassemble filter apparatus. Remember to empty the squirt bottle and secure all supplies. Your kit is ready to be used for the next sample run.

27 The 6" X 8" bag containing all of your filtered chlorophyll samples should remain on dry ice until it is delivered to the proper lab for processing. However, once flash frozen, it can be stored on wet ice.

28 Complete a lab chain-of-custody document (green sheet) for the batch of filters, and deliver with to the appropriate lab. This document can be custom designed, but must include data that details the project name, sample collector, date collected, station number and station name for each site, time collected, amount filtered, time filtered and time frozen for each sample, and include space for the lab to attach a printed label to. See current chlorophyll lab documents for example. Make a copy of the document for your records once receiving personnel have marked it with the time and date.

29 Holding time for chlorophyll *a* filters is 22 days. If your lab only processes in batches, it may be three weeks before the clock starts for a report to be generated. Values are usually expressed to the tenth.

30 Store all equipment and supplies in a safe and dry environment where they will be ready for use on the next field trip.

****Estimating photic zone using Secchi disk –** If a photometer is not available, a Sechhi 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. 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 your estimate of the photic zone depth.

It should be noted that using a Secchi disk is very subjective, and different staff 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.

Sample Handling

After collection, all sample handling should be minimized. Investigators should use 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.

J. Data and Records Management

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 obtain prior permission from first the Unit manager, and then the Program manager, and be documented accordingly.

K. Quality Assurance and Quality Control

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.

*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.

L. References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), 1992, *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, Washington, D.C.

APHA-AWWA-WEF, 1995, *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Washington, D.C.

APHA-AWWA-WEF, 1998, *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, Washington, D.C.

Georgia Department of Natural Resources, May 1990, *Safety Manual*, Atlanta, GA.

United States Environmental Protection Agency (USEPA), 1983, *Methods for Chemical Analyses of Water and Wastes*, Second Edition, Cincinnati, OH.

United States Environmental Protection Agency, Region IV, May 1996, *Environmental Investigations Standard Operating Procedures and Quality Assurance Manual*, Athens, Georgia.

United States Environmental Protection Agency, 1994, *NPDES Compliance Inspection Manual*, Washington, D.C.

United States Environmental Protection Agency (USEPA), Office of Environmental Information, April 2007, *Guidance for Preparing Standard Operating Procedures (SOPs)*, EPA/600/B-07-001, Washington, D.C.

United States Environmental Protection Agency (USEPA), Office of Water, March 1991, *Technical Support Document for Water Quality-based Toxics Control*, Second Edition, EPA/505/2-90-001, Washington, D.C.