

STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF CHLOROPHYLL-a SAMPLES IN LAKES

1.0 Introduction

The purpose of this document is to provide a simplified, step-by-step outline of the field and laboratory procedures used by the Water Quality Programs Division of the Oklahoma Water Resources Board (OWRB) for the collection of chlorophyll-a in lakes and reservoirs. The basic sampling procedures that will be discussed in this document involve water quality sampling, methods and equipment. All documents needed for, including chain of custody forms and laboratory login sheets for both the OWRB and the Oklahoma Department of Environmental Quality (ODEQ), field data sheets, and checklists can be found at the end of this document.

2.0 Definitions/Terms

3.0 Safety

Upon reaching the sampling location, site safety determinations should be made before proceeding. Please refer to the OWRB safety manual for information on boat safety, trailering and working from boats.

4.0 Quality of the Measurement

When sampling for all programs, Quality Assurance/Quality Control (QA/QC) samples will be routinely collected to assure that environmental samples meet the Data Quality Objectives (DQO's) that are outlined in the controlling Quality Assurance Project Plan (QAPP). QA/QC sampling is designed to control each step of the sampling process. Duplicate samples are collected to ensure that composite samples are properly processed. Replicate samples may be collected to ensure that the sampling methodology employed is collecting a representative sample. Spike or known samples may be submitted to test the efficacy of the analytical laboratory. The QA/QC protocols for sestonic chlorophyll-a can be found in the document "Standard Operating Procedure for the Collection of Water Quality Samples".

5.0 Personnel and Equipment

Principle investigators for the OWRB are required to have degrees and/or experience with biological or other applicable sciences. Principle investigators are defined as crew leaders, and this designation may be made upon the leader of a multi- or a one-person crew. Training is required for all SOPs dealing with water quality and quantity collections and measurements as well as habitat assessments and biological collections. In-house training will be conducted for the use of all meters and digital titrators used for water quality or quantity measurements. Investigators must be familiar with OWRB SOP document and all training will follow the methods outlined in that document. Extra training will be provided when new SOPs are developed. Training of field crews will be done through dry run exercises in the laboratory to familiarize field crews with sample collection, sample

preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods developed, qualified staff will train on sample collection, measurement, and field analysis methods through side-by-side field trips. These trips will familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will check field staff for adherence to SOPs.

Equipment used to collect the chlorophyll-a sample is described in the document “Standard Operating Procedure for the Collection of Water Quality Samples”.

5.1 Collection Equipment

The collection equipment is described in “Standard Operating Procedures for the Collection of Water Quality Samples”. When collecting samples, an additional clean 1-L sample bottle labeled for chlorophyll-a should be included.

5.2 Filtration Equipment

A field filtration unit should accompany a field crew when overnight travel is necessary. This unit is composed of a filtration apparatus, glass fiber or membrane filters (0.45 μm porosity, 47-mm diameter), rinse bottle, foil, marker, forceps, 250-mL plastic graduated cylinder, and zip-lock baggies. The filtration apparatus should include a glass filter funnel and base, a plastic or glass vacuum beaker (1000 mL), vacuum tubing, and hand pump. All glass and plastic parts should be thoroughly cleaned before leaving for the field. Vacuum tubing should be checked regularly for cracks, and the hand pump should be regularly checked to ensure that proper pressure can be regulated.

5.3 Extraction Equipment

Both chemical and mechanical extractions are used. For chemical extractions, a sufficient quantity of buffered acetone should be kept in supply. After chemical extractant is added, the sample is mechanically extracted either by manual use of a glass mortar and pestle or with an automated grinder. Extracted samples are placed in 15 mL screw cap vials. All extraction equipment should be cleaned thoroughly before and after each use.

6.0 Collection of Chlorophyll-a Samples

For chlorophyll-a sample collection, it is important to **prime the sample bottles** by rinsing the containers out with sample water before filling (fill the container with a little sample water, shake it, and pour the water out). Samples are collected by completely immersing the sample containers nozzle down (0.5 meters - approximately an elbow length below the surface) and slowly allowing sample container to fill. Avoid aerating the sample (i.e., don't allow water to “bubble” into the container). This can be accomplished by lowering the bottle in the lake with the nozzle down, allowing water to slowly fill the bottle, can accomplish this. **It is important to completely fill sample containers leaving no room for air in the container.** Cap sample bottle under water. Be sure to place the sample bottle in the ice chests immediately after collecting sample water. Typically, one (1) chlorophyll-a sample is collected per site. One extra sample is also collected at each lake as a QA/QC sample for chlorophyll-a comparison.

6.1 Extraction of Samples

Water collected for chlorophyll-a analysis will be processed immediately upon returning from the field (24-hour holding time). For BUMP lake crews, these steps may be completed before returning to the office in order to satisfy holding time requirements if overnight trips are required. Chlorophyll-a filtration **must be completed within 24 hours of sample collection**. Light and heat degrade chlorophyll, so it is imperative to minimize exposure to heat and sunlight and artificial light (i.e. don't process outside in direct sunlight, keep ice chest lids closed tightly). Chlorophyll-a must be filtered immediately after exposure to light to avoid degradation; therefore one bottle at a time should be removed from the refrigerator when ready to process samples. Chlorophyll-a filters must be frozen immediately after preparation and should be extracted for lab for analysis within one month of being processed. Log chlorophyll information on the appropriate log sheet. The chlorophyll log sheet is located at:

S:\Monitoring\BUMP\LAKES\FORMS\CHLORA LOG SHEET. The individual ODEQ lab sheets that accompany each individual vial of extracted chlorophyll is:

S:\Monitoring\BUMP\LAKES\FORMS\Chlora LAB.

Chlorophyll-a Sample Processing

Filtering

1. Assemble the bottom half of the chlorophyll filtering apparatus (everything but the clamp, filter paper, and graduated cylinder). Center a glass fiber or membrane filter (0.45 μm porosity, 47 mm diameter) on the filter base using forceps or a spatula. Clamp the graduated cylinder over the filter (make sure filter paper edges are covered by the graduated cylinder base). Dampen the filter paper with DI water and pump a few times to clear the graduated cylinder.
2. Rinse a graduated cylinder with a small amount of sample water. Measure a volume of sample water (start with 200 ml in turbid water, 300 ml in clearer water). **Be sure to mix the sample bottle well before pouring.** You may need to tap the bottom of the container to dislodge any settled particles. Note/record the initial water volume.
3. Filter the sample. The amount of water filtered is related to the turbidity of the sample. The more turbid the sample the less water you will be able to filter. Filter as much water as possible (but no more than 800 ml). Maintain hand pump pressure of less than 40 kPa (~ 10in Hg) to prevent cells from rupturing.
4. Record the final volume of water filtered on chlorophyll sample log sheet.
5. Rinse the inside of the graduated cylinder with DI water to include any remaining cells adhering to the sides. Pump the hand pump to clear the graduated cylinder and remove the clamp and well. With forceps or a spatula, fold the filter paper in half (topside in) being careful not to touch filtered material and remove the filter paper from the apparatus.
6. Filter paper may be ground immediately (**must** be ground immediately if in the field) or wrapped in aluminum foil, labeled (with lake name, site number, date, time collected, and volume filtered), and frozen until you or someone else has time to perform the grinding. If sample water has a pH > 7, the **filter** may be stored airtight (protected from light exposure) at 4 degrees Celsius for **three weeks**. Samples from acidic water, pH < 7, must be filtered and processed within 24 hours.

7. Insert new glass fiber filter into apparatus, wet with DI water, and filter next sample.

Grinding

1. Filter papers can be ground either by hand or with the mechanized tissue grinder. Place the folded filter paper in the mortar of the grinder and add approximately 2 cm **buffered** acetone. Chop up filter paper with a spatula, rinse spatula with acetone. Grind completely (no visible pieces of paper remain). Remember that light (sunlight and incandescent lamps) and heat degrade chlorophyll so be sure not to grind in direct sunlight (don't grind outside) and make sure the grinding process does not heat up the acetone mixture. This is especially important when using the mechanized grinder as it can heat up quickly. While grinding, be careful not to spill any of the acetone mixture. Should you spill, start over if possible. If not, make a note on the chlorophyll sample log sheet.
2. Pour acetone mixture into a 15 mL screw-cap chlorophyll tube labeled with Lake Name, site number, date collected, and volume filtered. Write on the label in pencil because acetone can wash away ink. Rinse mortar at least once completely with acetone and pour rinse into chlorophyll tube. Fill remainder of tube with buffered acetone, cap and store in the freezer until delivery to the lab. Be sure to copy the information on the label of the test tube onto the sample log sheet and note if a spill or breakage occurs.

7.0 Forms

7.1 Laboratory Log-in Sheets

Log-in sheets are documents turned into the analytical laboratory for each sample collected. These forms are used to denote the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time of sample collection and be legible and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. For guidance on proper procedure to complete the log-in sheets, refer to your supervisor and or FTE. Login sheets can be found at S:\Monitoring\BUMP\LAKES\FORMS\Chlora LAB.

7.2 Chains of Custody

Chains of custody are documents turned into the analytical laboratory for each group of samples collected. These forms are used for several purposes. They act as a legal document to show proper delivery of samples occurred and they make a general list of the parameters that should be analyzed. Chains of custody are available for inorganic, metals, and organics panels. They are a data sheet and should be treated as such. Therefore, they should include the date and time for each sample collected and be legible and complete. They should also be signed and dated by field and laboratory receiving personnel at the time of delivery. To avoid confusion and loss of data, a new chain of custody should be used for each group of samples. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and or FTE. Chains of custody can be found at S:\Monitoring\BUMP\LAKES\FORMS\CHLORA LOG SHEET.

8.0 Data Storage

All completed paper copies of forms and data sheets should be maintained with the appropriate station notebook. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Water Quality Database. Each sample should be maintained electronically in the database under a unique sample number.

9.0 References

American Public Health Association, et. al. Standard Methods for the Examination of Water and Wastewater (18th ed.). Port City Press, Baltimore, MD., 1992.

Cole, Gerald. Textbook of Limnology. 3rd ed. Illinois: Waveland, 1983.

Oklahoma Department of Environmental Quality. Continuing Planning Process. 1994 ed. unpublished.

FOR LAB USE ONLY:
PROJECT CODE: WB-CL
SELS SAMPLE NO.

**OKLAHOMA DEPARTMENT OF ENVIRONMENTAL QUALITY
STATE ENVIRONMENTAL LABORATORY
707 N. ROBINSON
OKLAHOMA CITY, OK 73102
(405) 702-1113
OKLAHOMA WATER RESOURCES BOARD
CHLOROPHYL LOGIN FORM**

Collector: _____ Lake: _____

Date Collected: _____ Time Collected: _____

Site #: _____ Site ID#: _____

Extraction Date: _____ Extractor's Initials: _____

Volume Filtered: _____ ml. Date Filtered: _____

QA Code: _____

Comments: _____

Corrected Chlorophyll A (32210)

Pheophytin A (32218)

Return To: OWRB – Water Quality
Julie Chambers
3800 N. Classen Blvd.
Oklahoma City, OK 73118

Copy: File Copy

**CHLOROPHYLL SAMPLE CHAIN OF CUSTODY – BUMP LAKES – PROJECT CODE: WB-CL
OKLAHOMA WATER RESOURCES BOARD**

Lake Name	Site #	Date Collected	Time Collect ed	Collector Initials	Volume Filtered (mL)	Date filtered	Date Extracted & Initials	Comments	Sample #

Lake Name	Site #	Date Collected	Time Collected	Collector Initials	Volume Filtered (mL)	Date filtered	Date Extracted & Initials	Comments	Sample #
Relinquished by: OWRB					Date Relinquished:				
Signature:					Time Relinquished:				
Receiving Organization: ODEQ Laboratory					Date & Time Received:				
Person Receiving: Signature					Comments:				

Return to: Julie Chambers
OWRB
Water Quality Programs Division
3800 N. Classen Blvd.
Oklahoma City, OK 73118