Standard Operating Procedure for the Collection and Processing of Chlorophyll-a Samples in Lakes

Revised and Adopted July 2018

Final Copy

OKLAHOMA WATER RESOURCES BOARD
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Standard Operating Procedure for the Collection and Processing of Chlorophyll-a Samples in Lakes

November 2019

Revision Date	Version	Description of Changes	Effective Date
July 2018	1.1	• Updated steeping hold time to analysis within 4-6 hours • Addition of recording extraction time and date on field sheet and Chain of Custody	September 2019
Final	2.0	• Finalized edits • Updated format	November 2019

STANDARD OPERATING PROCEDURE FOR THE COLLECTION AND PROCESSING OF CHLOROPHYLL-a SAMPLES

Originally Adopted 2013

Revised July 2018

1.0 Introduction

The purpose of this document is to provide an outline of the chlorophyll-a sample collection and processing procedures used by the Water Quality Programs Division of the Oklahoma Water Resources Board (OWRB) for all projects that are part of the Lakes Monitoring Program. Techniques for sampling will be outlined in this document; an experienced staff member will conduct further training on an as-needed basis. An example of documents pertaining to lakes sampling, including chain of custody form for Oklahoma Department of Environmental Quality (ODEQ) and field data sheets are appended 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 operating on boats (OWRB, 2017).

4.0 Quality of the Measurement

A variety of Quality Assurance/Quality Control (QA/QC) samples are routinely collected to assure that environmental samples meet the Data Quality Objectives (DQO's) outlined in the controlling Quality Assurance Project Plan (QAPP). QA/QC sampling is designed to control each step of the sampling process. The project QAPP should be consulted before trip planning to ensure the appropriate samples are collected. Blank samples are collected to ensure that field personnel are effectively cleaning the equipment used in field sampling, laboratory cleaning methods are adequate, and deionized water is clear of impurities. Duplicate samples may be collected to ensure that composite samples are properly homogenized and processed. Replicate samples may be collected to ensure that the sampling methodology employed is collecting a representative, repeatable sample. Spike or known samples may be submitted to test the efficacy of the analytical laboratory.

5.0 Personnel and Equipment

5.1 Personnel

Principal investigators for the OWRB are required to have bachelor degrees and/or experience with biological or other applicable sciences. Each sampling trip will have a designated crew leader along with other investigators. In all instances, the collection of water quality samples from lakes requires at least a two-person field crew. Investigators must be familiar with OWRB SOP documents concerning 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. Training will follow the methods outlined in these documents; additional training will be provided when new SOPs are developed. Field crew training will be accomplished through dry-run exercises in the laboratory to familiarize crews with sample collection, sample preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods are developed, qualified staff will train them on sample collection, measurement, and field analysis methods via side-by-side field trips to familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will audit field staff for adherence to SOPs.

5.2 Equipment

In addition to regular sampling bottles, a clean 1-L amber sample bottle labeled for chlorophyll-a should be included for each site and for each QA code where chlorophyll is collected. Sample bottles should be labeled on the lid and body of the amber bottle.

5.2.1 Filtration Equipment

A field filtration kit should accompany a field crew when overnight travel is necessary. This kit is composed of a filtration apparatus, glass fiber or membrane filters (0.45µm porosity, 47-mm diameter), rinse bottle, foil, marker, forceps, 250-mL plastic graduated cylinder, and zip-lock bags. 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 with deionized (DI) water 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.

5.2.2 Extraction Equipment

Both chemical and mechanical extractions are used to prepare chlorophyll samples for analysis. For chemical extractions, a sufficient quantity of buffered acetone should be kept in supply. After chemical extractant is added, the sample is mechanically extracted by manual grinding using a glass mortar and pestle. Extracted samples are poured in 15 mL screw cap vials and wrapped in foil to limit light exposure. All extraction equipment should be cleaned thoroughly before and after each use. To ensure cleanliness, rinse all equipment with DI and acetone before and after extraction.

6.0 Collection of Chlorophyll Samples

For chlorophyll-a sample collection, it is important to first **prime the sample bottles three times** by rinsing the containers with ambient 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 container nozzle down to a depth of 0.5 meters (approximately an elbow length below the surface) and slowly allowing sample container to fill. Avoid aerating the sample, by lowering the bottle nozzle-down, allowing water to fill the bottle slowly. **It is important to completely fill sample containers leaving no room for air in the container.** Cap sample bottle under water. Place the sample bottle on ice immediately after collecting sample. Typically, one chlorophyll-a sample is collected per site. One extra QA/QC sample is also collected at each lake, typically at site 1, for chlorophyll-a comparison.

6.1 Chlorophyll Sample Processing

Water collected for chlorophyll-a analysis must be filtered as soon as possible, as these samples have a 24-hour holding time. If overnight trips are required, these steps should be completed before returning to the office in order to satisfy holding time requirements. Light and heat degrade chlorophyll, therefore it is imperative to minimize exposure to heat, sunlight, and artificial light. Do not process samples in direct or incandescent light and keep ice chest lids closed tightly. Chlorophyll-a must be filtered immediately after exposure to light and be kept under 4^0 C; therefore only one or two bottles should be removed from the cooler or refrigerator at a time. Chlorophyll-a filters may be frozen immediately after preparation and should be extracted for lab analysis as soon as possible. If unable to process immediately, the hold time is twenty-eight days; however this is the exception and should only be lengthened to this time in extenuating circumstances. Once samples have been extracted and are in the tubes, the steeping process has begun. **Samples need to be analyzed at the lab within 4-6 hours**, so be courteous and take samples to the lab as early in the morning as possible.

6.1.1 Filtering Chlorophyll Samples

1. Assemble the bottom half of the chlorophyll filtering apparatus, the vacuum flask and filter base, using the following steps. Center a glass fiber or membrane filter (0.45 μm porosity, 47 mm diameter) smooth side down on the filter base using forceps or a spatula. Clamp the graduated filter cup over the filter (make sure filter is covered completely). Dampen the filter paper with DI water and pump a few times to clear the graduated filter cup.
2. Prime a graduated cylinder with a small amount of sample water. Gently homogenize the sample water before pouring, by inverting the bottle several times to create a uniform mixture. You may need to tap the bottom of the container to dislodge any settled particles. Measure a volume of sample water (start with 200 ml in turbid water, 300 ml in clearer water). Record the initial water volume, measured from the bottom of the meniscus.
3. Filter the sample. The amount of water able to be 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 750 mL. Maintain pump pressure of no more than 7 inches Hg (3.4 psi) to prevent cells from rupturing and to not overload filters. Make sure the filter is dry, but do not over-dry.
4. Record the final volume of filtered water on field sheet and chain of custody (CoC). The same volume of water should be filtered for the environmental sample and its associated replicates and duplicates.
5. Rinse the inside of the filter cup with DI water to include any remaining cells adhering to the sides. Pump to clear the graduated filter cup and remove the clamp. With forceps or a spatula, fold the filter paper in quarters, being careful not to touch filtered material and remove the filter paper from the apparatus.
6. Filter paper may be ground immediately or wrapped in aluminum foil, labeled (with lake name, site number, date, and volume filtered), and frozen. Samples should be extracted as soon as possible, but be cognizant of the 4-6 hour time window to submit samples to laboratory.
7. Rinse filter cup, filter base, graduated cylinders, forceps and spatula with DI water. Repeat steps 1-7 until all samples are filtered.

6.1.2 Extracting Chlorophyll Samples

1. If the acetone pump is available, simply pump buffered acetone into squeeze bottle. If the acetone pump is not available, decant buffered acetone into an empty amber jug in order to keep magnesium carbonate from resuspension. Fill squeeze bottle with the decanted buffered acetone, taking care to limit any remaining buffer from entering the bottle.
2. Rinse grinding tube, pestle, and spatula with buffered acetone.
3. Filter papers are extracted (ground) by hand. Place the folded filter paper in the grinding tube and add approximately 5 mL buffered acetone. Macerate filter paper with a spatula, rinse spatula with buffered acetone into the mortar.
4. Grind completely with a snug-fitting pestle until no visible pieces of paper or algal material remain. Remember that light (sunlight and incandescent light) and heat degrade chlorophyll; therefore, **be sure not to grind in direct sunlight.** While grinding, be careful not to spill any of the acetone mixture. Should you spill, start over with a newly filtered sample, if possible. If not, make a note of how much volume was lost on the field sheet and chain of custody.
5. When removing pestle from the grinding tube, rinse it and the walls of the tube with acetone to rinse any remaining residue into the extracted volume.
6. Pour extracted sample into a 15 mL screw-cap chlorophyll vial. Rinse grinding tube with acetone and pour rinsate into chlorophyll vial, taking care not to overfill. Fill remainder of vial with buffered acetone and screw on lid.
7. Place the appropriate bar code label vertically on the vial as close to the top as possible. Cover with foil, and store on ice or in the freezer until delivery to the lab. Record all required information on the chain of custody and field sheet, documenting if a spill or breakage occurs in the samplers comment section.

8. Once samples have been extracted, the steeping process has begun. **Samples need to be analyzed at the lab within 4-6 hours** of grinding, so be courteous and take samples over as early in the morning as possible.
9. Thoroughly clean the pestle, grinding tube, and spatula by rinsing with DI water and rinsing a final time with acetone. Carefully inspect the equipment for any residue that may be adhering and remove with a clean tissue.
10. Proceed to the next filter and repeat the steps above.

7.0 Forms

7.1 Chains of Custody

Chains of custody are documents remitted to the analytical laboratory documenting characteristics and metadata for each lake's samples. 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. They are a data sheet and a legal document and should be treated with the responsibility this dictates. The date, time, and filter volume for each sample collected must be included and the form should be legible, accurate, and complete. Additional information such as chlorophyll–a extraction date and time, acid, and bottle lot numbers should also be included. All forms should be signed and dated by field collector and laboratory receiving personnel at the time of delivery. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and/or crew leader. All samples are pre-logged in advance of sampling and a chain of custody document is generated by the ODEQ. The chain of custody is included with the trip sample kit that is picked up by field staff the week prior to the sampling trip. If bacteria or extracted chlorophyll samples are to be turned in at a later date a new chain is generated and remitted with those samples.

8.0 Data Storage

When weather permits, the electronic field sheet should be completed on the laptop and saved in the format "Sample ID Lake Name Season Year." If the paper copy is used, it should be stored in the appropriate binder. The data from the electronic field sheet should be transferred to the network upon arrival to the office for review and upload to the Ambient Water Quality Monitoring System (AWQMS). Each sample should be maintained electronically in the database under its unique sample number.

9.0 References

American Public Health Association, et. al. (2013). *Standard Methods for the Examination of Water and Wastewater, 22nd edition.* Baltimore, MD: Port City Press.

Cole, G. (1994). *Textbook of Limnology, 4th edition.* Illinois: Waveland Press, Inc.

ODEQ. (2012). *Continuing Planning Process.* Oklahoma City: ODEQ.

OWRB. (2017). *Office and Field Safety Manual.* Oklahoma City: OWRB.

OWRB. (2018). *Standard Operating Procedure for the Collection of Water Quality Samples in Lakes.* Oklahoma City: OWRB.

U.S. Environmental Protection Agency. (1997). *Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence.* Cincinnati, Ohio: National Exposure Research Laboratory, Office of Research and Development.

U.S. Environmental Protection Agency. (1997). *Method 446.0: In Vitro Determination of Chlorophylls a, b, c +c and Pheopigments in 1 2 Marine and Freshwater Algae by Visible Spectrophotometry.* Cincinnati, Ohio: National Exposure Research Laboratory, Office of Research and Development.

Bump Lakes Field Data Sheet

Oklahoma Water Resources Board

Lake/Reservoir Data:	Data Collectors:	Instrument Data:
Reservoir Name:	Water:	Sonde #:
OWRB Trip ID Number: 1000	Sonde/Env:	Handheld #:
Visit Date & Quarter:	Additional:	Amber Bottle Lot:
Lake Elevation:	Van Dorn ID: Churn ID:	Clear Bottle Lot:
	Int Sampler: Project:	Acid Lot:
		DI Date:
		DI Lot #: 235

General Information

Site #	Time (24 Hr)	Air Temp (°C)	Wind Direction*	Avg. Wind Speed (MPH)	Cloud Cover % 0-25-50-75-100	Precipitation*	Wave Condition*	Barometric Pressure (mmHg)	Spc. Cond (µS/cm)	Site Depth (m)	Secchi Depth (cm)	Zoo Sample (m)
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
33												

Site/Lake Comments

Bump Lakes Field Data Sheet

Oklahoma Water Resources Board

Site #	Alkalinity (mg/L)	Hardness (mg/L)	Turbidity (NTU)	Chl-a Volume (mL)	Chlorophyll Extractor Initials:
1(12)					
1(22)					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
(33)					
Dilution %					
Titrant:					

Chemistry Times

Date & Time

Chemistry Started: _____

Chlorophyll Filtered: _____

Chlorophyll Extracted: _____

Field Sheet Review

Date & Time

Fieldsheet Completed: _____

Office Reviewed: _____

Uploaded To AWQMS: _____

Chemistry Notes/Comments

_____

_____

_____

_____

_____

_____

_____

_____

STATE ENVIRONMENTAL LABORATORY SERVICES DIVISION
CHAIN OF CUSTODY

General Inquiries (toll free): 1-866-412-3057

PROJECT INFORMATION

Contact Name: OWRB	Samplers (Print Names): ZMM LAD	Project Number: (Lab Use Only) OWRB-001_0619
Trip Name: HEYBURN		

SAMPLE INFORMATION / **TESTING REQUIRED**

Sample #	Description	Date Collected (MM/DY)	Time Collected (Circle AM/PM)	Sample Depth	Sample Matrix [1]	Container [2]	(Waters) Collection Type [3]	QA Code	Thermal Pres.	TESTING REQUIRED
OWRB-1301225-01	1000496.01A Heyburn (01S)	9/27/18	955 AM PM	0.5	AQU	CPB 1L H2SO4	SRF	12	Ice	NO2 NO3, TKN, TOTAL P
OWRB-1301225-02	1000496.01B Heyburn (01S)		AM PM	0.5	AQU	CPB 1L NONE	SRF	12	Ice	TDS, CHLORIDE, SULFATE, Conductivity 201.3
OWRB-1301225-03	1000496.01C Heyburn (01S)		AM PM	0.5	AQU	CGT 12ML NONE	SRF	12	Ice	CHLOROPHYLL, Filter Volume 250
OWRB-1301226-01	1000496.01D Heyburn (01S)		AM PM	0.5	AQU	CPB 1L H2SO4	SRF	22	Ice	NO2 NO3, TKN, TOTAL P
OWRB-1301226-02	1000496.01E Heyburn (01S)		AM PM	0.5	AQU	CPB 1L NONE	SRF	22	Ice	TDS, CHLORIDE, SULFATE, Conductivity 201.3
OWRB-1301226-03	1000496.01F Heyburn (01S)		955 AM PM	0.5	AQU	CGT 12ML NONE	SRF	22	Ice	CHLOROPHYLL, Filter Volume 250
OWRB-1301227-01	1000496.02A Heyburn (02)		1115 AM PM	0.5	AQU	CPB 1L H2SO4	SRF	10	Ice	NO2 NO3, TKN, TOTAL P
OWRB-1301227-02	1000496.02B Heyburn (02)		1115 AM PM	0.5	AQU	CPB 1L NONE	SRF	10	Ice	TDS, CHLORIDE, SULFATE, Conductivity 203.1
OWRB-1301227-03	1000496.02C Heyburn (02)		1115 AM PM	0.5	AQU	CGT 12ML NONE	SRF	10	Ice	CHLOROPHYLL, Filter Volume 250

SAMPLER'S COMMENTS

SAMPLE RECEIVING COMMENTS

CHAIN OF CUSTODY RECORD MUST BE SIGNED

*** SEE PAGE 2 FOR CHAIN OF CUSTODY RECORD ***

707 NORTH ROBINSON, P.O. BOX 1677, OKLAHOMA CITY, OKLAHOMA 73101-1677