
WATER QUALITY PROGRAMS DIVISION

Standard Operating Procedure for the Collection of Benthic and
Sestonic Chlorophyll-a Samples in Streams

Revised and Adopted May 2004

Draft Copy



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STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF BENTHIC AND SESTONIC CHLOROPHYLL-a SAMPLES IN STREAMS

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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 benthic and sestonic chlorophyll-a in wadeable rivers and streams. 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. These will be different for wadeable and bridge sites. Please refer to the OWRB safety manual for instructions on how to sample both kinds of sites. When regulating the flow of traffic is necessary, please refer to the portion of the safety manual outlining "Traffic Safety Protocols".

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. Blanks are collected to ensure that field personnel are properly cleaning the plastics and glassware used in field sampling. 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". The QA/QC samples for benthic chlorophyll-a are the same. However, since both collection and filtration equipment are both used more than once in the field, two code "33" samples should be taken—one for collection equipment and one for filtration equipment.

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.

In most instances, the collection of water quality samples requires only one field person. However, depending on the safety requirements of a particular station, additional crewmembers may be necessary to ensure a safe work zone. Equipment used to collect the chlorophyll-a sample are described in the document “Standard Operating Procedure for the Collection of Water Quality Samples”.

5.1 Collection Equipment

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

The sestonic sample is merely collected from the splitter churn as an additional composite sample. To ensure cross-contamination has not occurred, a field blank (QA code 33) should be processed when sestonic chlorophyll-a samples are collected.

For benthic samples, the field collection unit should accompany the field crew. This unit includes a 1 or 2 gallon calibrated wide mouth jug, a large funnel, hard and soft substrate delimiters, coarse scrubbing brush, knife, spatula, rinse bottle, hip chain (extra string and stake), camera, and calculator. All parts should be cleaned thoroughly before leaving the office and while in the field. To ensure cleanliness, both laboratory (code 32) and field (code 33) blanks should be collected using all equipment coming into contact with the sample.

5.2 Filtration Equipment

A field filtration unit should accompany a field crew when benthic and sestonic chlorophyll-a collections are being made. The unit should be cleaned thoroughly after each use. This unit 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 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. To ensure cleanliness, a laboratory blank (QA code 32) should be filtered and processed. 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

For benthic samples, only chemical extraction is used. A clean and labeled 100 mL polyethylene sample bottle should be included for each sample. Before leaving for the field, each bottle should be filled with 25 mL of reagent grade ethanol, tightly capped, and marked along the fill line. Before use in extraction, the line should be checked to ensure that no ethanol has spilled or evaporated.

For sestonic samples, both chemical and mechanical extraction 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 13 mL screw cap vials. All extraction equipment should be cleaned thoroughly before and after each use, a laboratory blank (code 32) should be collected when samples are processed.

6.0 Collection of Chlorophyll-a Samples

6.1 Benthic Sampling

Following is a detailed description of sampling procedures. Because sampling sequence is important, please follow the protocol as outlined. The general methodology underpinning periphyton sampling involves collecting and compositing samples taken at equidistant transects along a representative reach. Within this reach, samples will be collected in several representative habitats—erosional and depositional. Erosional habitats include riffles and runs.

Depositional habitats are slack water and are mostly contained within pooling areas. In order to collect a representative sample within each stream reach, each type of habitat should be sampled. The sampling sequence will include the following generalized steps:

1. Establishment of reach and transects
2. Collection of samples
3. Extraction of samples

6.11 Establishment of reach and transects

The stream reach is defined as 20 times the wetted width of the widest section. Along this reach, 11 equidistant transects are sampled in an effort to sample all represented habitats. To establish, follow these steps:

- a. To establish the sampled stream reach, measure the widest wetted width in meters and multiply by 20 (e.g., widest wetted width = 11 meters; stream reach = 20×11 meters = 220 meters).
- b. To establish 11 equidistant transects (A-K), divide the total stream reach by 10 (e.g., stream reach = 220 meters; transect width = $220 \text{ meters} / 10 = 22$ meters). Using the previous example, transect A will be at the head of the stream reach (0 meters), transects B-J will be at 22 meter intervals, and transect K will be at the bottom of the stream reach (220 meters).

6.12 Collection of Samples

At each transect, the sample will be collected at left (L), right (R), or center (C). The type of substrate—soft or hard, will determine the method used to collect the sample. Soft substrates

include sand, silt and gravel. Hard substrates are all materials larger than gravel including hardpan and bedrock. **Please keep composited sample out of sunlight.** To collect, follow these steps:

- a. The sampling point for each transect is established randomly at the first transect. Several methods may be used to randomize the first point, but the most available method involves the second hand on a watch. By looking at the second hand, “L” is represented by 1-20, “C” by 21-40, and “R” by 41-60. Each following sampling point is established by going from left bank to right bank, back to the left bank and on to the right bank. Repeat this process until all transects are assigned a sampling point.
- b. Sampling moving upstream, determine the most representative habitat type at each transect and sample that habitat. Sampling technique will be dependent upon substrate type. Always pick an accessible collection area along transect. Collection area should be as close to the center of the sampling point as possible. Do not sample depths deeper than mid-bicep. Composite samples from each point into 1 or 2-gallon wide mouth jar.
- c. Sampling Soft Substrates
 - Place capped delimiter over substrate, pressing in until pressure is felt on cap.
 - Slide spatula underneath delimiter and lift sample from water ensuring that none of the sample is lost.
 - Pour sample into 1-gallon jar using small funnel. Using native water, rinse the delimiter, spatula and funnel.
- d. Sampling Removable Hard Substrates (e.g., cobble)
 - Remove rock(s) from stream and place open delimiter over substrate to define sampling area. Outline sampling area with a sharp edge.
 - Place rock(s) into large funnel and scrub delimited area with a medium coarse wire brush. Periodically wash scrubbed area into jar and continue scrubbing until all periphyton has been removed from rock.
- e. Sampling Unremoveable Hard Substrates (e.g., hardpan)
 - Place capped delimiter over substrate, pressing in until pressure is felt on cap.
 - Slide spatula underneath delimiter and lift sample from water ensuring that none of the sample is lost.
 - Pour sample into 1-gallon jar using small funnel. Using native water, rinse the delimiter, spatula and funnel.

6.13 Extraction of Samples

Extraction method is 48 hours in ethanol at ambient temperature. Mechanical extraction is not used. The following steps are used to extract the subsample.

- A subsample of 25 mL from a completely mixed composite is measured using a graduated cylinder.
- The subsample is filtered using a glass or fiber filter (0.45µm porosity, 47-mm diameter). Filtration should not occur above 20 psi.
- When entire subsample is filtered, the filter is removed from the unit and placed in 25 mL of ethanol. The ethanol should be in a wrapped 100 mL polyethylene bottle.

- Sample is transported in the cab of the truck and should be delivered to the laboratory within 48 hours.

6.2 Sestonic Samples

Sestonic chlorophyll sampling and post-processing for streams is described in the lakes portion of the monitoring SOP. For streams, the only sampling difference will be the collection of a composited, depth-integrated sample from the splitter churn. This will be done while general chemistry samples are being aliquoted. Water collected for chlorophyll-a analysis has a 24 hour holding time and should be processed immediately in the field. 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. Chlorophyll-a filtrates must be wrapped in foil, labeled, bagged and frozen on ice immediately upon processing. These filtrates may be kept frozen for up to 30 days before extraction occurs. Extracts must also be frozen immediately after preparation and should be submitted to the lab for analysis within one month of being processed.

6.3 Photo Documentation

7.0 Forms

7.1 Field Notes

Field notes are documents used to annotate and record information that is gathered at the project site. They are a data sheet and should be treated as such. Therefore, they should be written, legible, and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. Field notes should be initialed and dated by the collecting personnel and data entry personnel. For guidance on proper procedure to complete the field notes, refer to your supervisor and or FTE. Field notes can be found at S:\Monitoring\STREAMS\forms\Field Notes.doc.

7.2 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. Log-in sheets can be found at S:\Monitoring\STREAMS\forms\.

7.3 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\STREAMS\forms\.

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

Hill, B. H., "Periphyton", EMAP Western Pilot Study Field Operations Manual for Wadeable Streams, 156-158 (2001).