WATER QUALITY PROGRAMS DIVISION

Standard Operating Procedure for the Collection, Filtration, and Extraction of Benthic and Sestonic Chlorophyll-a Samples

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OKLAHOMA
Water Resources Board

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STANDARD OPERATING PROCEDURE FOR THE COLLECTION, FILTRATION, AND EXTRACTION OF BENTHIC AND SESTONIC CHLOROPHYLL-a SAMPLES

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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*. The basic sampling procedures that will be discussed in this document involve water quality sampling, methods and equipment.

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 all sites. Please refer to the OWRB safety manual for instructions on how to safely access sample various 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.

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 two field personnel. 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 samples 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 dark sample bottle labeled for chlorophyll-a should be included. 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. For each site, this unit includes a delimiter with a neoprene collar, brush, aspirator, squeeze bottle, 5-40mL screw cap vials, and camera. All parts should be cleaned thoroughly before leaving the office and between each site 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. Also all mailer equipment should be taken on trip including ice chest, quart size zip-lock bags (2 per sample), shipping tape, and shipping label.

5.2 Filtration Equipment

A field filtration unit should accompany a field crew when 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 could be regulated. Crews should also maintain a large bottle with dry desiccant for each trip.

Benthic samples are typically filtered by the contract laboratory. If filtration is required in the field, the same equipment and procedures as described in sestonic sampling are used. The only difference is that a much smaller amount of sample will be filtered.

5.3 Extraction Equipment

For sestonic samples, 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.

Benthic samples are typically extracted by the contract laboratory. If extraction is required, the same equipment and procedures as described in sestonic sampling are used.

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 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. Sample Shipping
4. Sample filtration and extraction

6.11 Establishment of reach and transects

The stream reach is defined as 400 to 800 meters depending on the size and characteristics of the stream. Choosing the reach length is more clearly defined in the Biological Collection SOP's. Along this reach, 5 equidistant transects are sampled in an effort to sample all represented habitats. To establish, follow these steps:

a. To establish 5 equidistant transects (A-E), divide the total stream reach by 5(e.g., stream reach = 400 meters; transect width = 400 meters/5 = 80 meters).
b. Using the previous example, transect A will be at the head of the stream reach (0 meters), transects B-D will be at 80 meter intervals, and transect E will be at the bottom of the stream reach (400 meters).

6.12 Collection of Samples

Starting at the downstream transect A, randomly select a bank to begin from. If sampling begins on the right bank for transect A, move to the left bank for transect B, the right bank for transect C, etc. until all transects are sampled. The goal is to collect five benthic periphyton samples for each stream reach.

Once the periphyton sampling bank (left or right) has been determined, select a suitable substrate for sampling within 1 m up or downstream from the transect line, and no more than 25% of the stream width away from the bank. The sample substrate need not be the "dominant substrate" that is found in the transect. For analysis purposes, it is very important to identify the periphyton sample substrate types. The Periphyton Field Form characterizes substrates in five categories including: Rock/cobble, Sand/gravel, Mud/silt, Wood, Leaf, and Other. Mud/silt substrates (not gritty) should be distinguished from Sand/Gravel substrates (gritty to tennis ball size), while any Rock/Cobble substrates larger than coarse gravel (larger than a tennis ball) should be categorized as such. If necessary, samples may also be taken from submerged Wood, Leaf material, or from some other substrate (*e.g.*, trash), but should be noted on the Periphyton Field Form.

For sampling, follow one of the two procedures below. Fit the aspirator apparatus firmly into the mouth of the sample vial and then follow the appropriate procedure below. For either procedure, should the delimiter become dislodged during the process, discard the sample, rinse the brush, delimiter, and sample vial with rinse water from the squeeze bottle, and repeat the sampling process as described. The vial need not be filled completely. Take great care not to overfill the vials: the aspirators do not have an overflow valve, so overfilling can result in an undesirable mouthful of stream water for the sampler. Place collected samples on ice in a dark cooler. Be sure to record on the Periphyton Sampling Form the transect, bank, and substrate from which each sample was taken. To collect, follow one of these methods:

For solid substrates (with available flat surface ≥3.5 cm diameter, e.g., cobble, wood)

a. When water near the edge is deep and/or turbid. The substrate may be lifted out of the water and sampled on shore. Use common sense to select a substrate that is within the photic zone.
b. Place the delimiter firmly on the substrate (to form a seal) and hold in place throughout sampling.
c. Squirt a small quantity of rinse water from the squeeze bottle directly onto the substrate (10-15 mL, or about to the top of the black neoprene collar).
d. Scrub with the brush to loosen any attached periphyton, and aspirate the loose particles into the sample vial.
e. Rinse the brush and delimiter.
f. Aspirate two more times to get additional material.

For soft or small substrates (e.g., silt, sand):

a. Select an undisturbed sample site at least 1 cm below the water surface, but be careful not to sample in water deeper than the delimiter will allow (about 8 cm). Use common sense to select a substrate that is within the photic zone.
b. Place the delimiter gently onto the substrate, and slowly press it down into the substrate to form as much of a seal as possible (without disturbing periphyton in the 3.5-cm diameter sample space).
c. Put the tip of the aspirator as close to the substrate as possible and, sweeping back and forth across the sample space, vacuum up the entire surface layer.
d. Aspirate as much as possible, up to the sample vial volume (40 mL).
e. Use additional rinse water from the squeeze bottle if necessary.

It is very important to keep samples cold and in the dark at all times. Between sites, be sure to clean and rinse the aspirator and brush with deionized water and allow to air dry. Periodically clean the aspirator apparatus with ethanol (especially the mouthpiece, for sanitary reasons), rinse well with clean water, and allow to air dry.

6.13 Sample Shipping

Place the five 40-mL sample vials into two quart-size ziplock plastic bag, and seal. Multiple sites may be shipped in the same mailer but must be packaged in baggies separately. Place this bag and the one 250-mL plastic bottle (if sestonic sample is to be collected and included for the study) in the provided cooler, and pack with bagged ice sufficient to reach the contract lab. Pack securely to prevent breakage of vials and leakage of water. Clearly mark any bags of ice as ice. Place the original signed, chain of custody and photocopies of the Periphyton Field Form(s) in another sealed ziplock plastic bag and tape to the underside of the cooler lid. Secure the cooler with strapping tape, affix the provided shipping label, and ship to the contract lab within 48 hours of field collection. Call 1-800-463-3339 to arrange FedEx pickup or find the nearest service center for dropoff. Telephone the lab below to notify them that the sample was shipped. If recording a voicemail, leave your name, agency name, contact number, date and time, and number of coolers shipped.

6.14 Filtration and Extraction of Samples

Benthic samples are typically filtered and extracted by the contract laboratory. If extraction is required, the same equipment and procedures as described in sestonic sampling are used. The only difference is that a much smaller amount of sample will be filtered.

6.2 Sestonic Samples

Following is a detailed description of sampling procedures. Because sampling sequence is important, please follow the protocol as outlined. The sampling sequence will include the following generalized steps:

1. Sample Collection
2. Sample Filtration
3. Sample Extraction (program dependant)

6.21 Sample Collection

Sestonic chlorophyll samples are collected using the most appropriate method of collection as found in "Standard Operating Procedures for the Collection of Water Quality Samples". Determining appropriate sample methodology is fully described in the SOP for the Collection of Water Quality Samples. Water collected for chlorophyll-a analysis has a 24-hour holding time before filtration and should be processed immediately in the field.

6.22 Sample Filtration

Chlorophyll-a must be filtered immediately after exposure to light to avoid degradation. Filtrates may be kept frozen for up to 30 days before extraction occurs. To filter sample do the following:

1. Set up the filtering funnel by putting the base of the funnel into the flask. Place a glass fiber filter on the screen in the funnel base. Place the funnel on top of the base. Using the funnel clamp, clamp the funnel and base together.
2. After mixing the sample completely, measure the water to be filtered in a graduated cylinder. Filter the water through the glass fiber filter, using suction (do not exceed -20 kPa). Continue to filter until the rate of flow through the filter decreases markedly.
3. When enough water has been filtered, stop suction, and remove the funnel top.
4. Remove the filter and fold it in half with the chlorophyll side inside. Blot the filter dry on a paper towel, and clip it into a filter paper wrapper on which is recorded: Station, date, volume of water filtered, and initials of sampler.
5. Store the samples in a closed jar with silica gel desiccant in an ice chest with ice or the freezer.

6.23 Sample Extraction

Extraction will be done in the OWRB laboratory. Equipment will include screw-capped glass test tubes, 90% ethanol, forceps, water bath, and a test tube rack. Extraction of chlorophyll-a pigments will follow modified procedures of Sartory and Grobbelaar (1984) and Canfield et al (2002). It is important during each step of extraction to keep samples away from light. To extract sample do the following:

1. Place the folded filters in 13 mL screw cap glass vials.
2. Add 8.0 mL of 90% ethanol and cap the tubes. The 8.0 mL amount allows a head space at the top of the vial for heating.
3. Place the rack of tubes in a water bath heated to 78^{o}C (172 ^{o} F). Heat the tubes for 5 min. Begin timing after the water bath returns to 78 ^{o}C.
4. Place the tubes in a dark cabinet at room temperature and allow to stand in the dark for 24 hr.
5. Take samples to the contract laboratory for spectrophotometric analysis.

6.3 Photo Documentation

Photo documentation is an important part of all water quality sampling. For chlorophyll sampling, photos may be used to assist in standards implementation. Pictures can be used to compare chlorophyll-a biomass to “aesthetic” properties of the waterbody. It is important to consistently photo document each transect of a benthic sample as well as general stream conditions when collecting both benthic and algal samples.

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 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.

8.0 Data Storage

All completed paper copies of forms and data sheets should be maintained with the appropriate network folder. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Ambient Water Quality Monitoring System (AWQMS). Each sample should be maintained electronically in the database under a unique sample number.

9.0 References

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