Standard Operating Procedure

Collection methods for benthic algae in wadeable waters

Commonwealth of Kentucky
Energy and Environment Cabinet
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Division of Water

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4. PROCEDURES

4a. Scope and Applicability

This SOP describes field methods for collecting benthic algae from wadeable waters (streams and near shore areas of lakes and rivers). The procedures are performed by the Monitoring Section of the Water Quality Branch (WQB), for bioassessment programs and special studies. The sampling methods are intended to be applicable for a wide range of waterbody types to allow broad applicability. The specific method chosen will vary depending on project objectives (see section 4b).

This SOP contains the methods in routine use by WQB, and have been extracted and edited from the large methods manual "Methods for Assessing Biological Integrity of Surface Waters in Kentucky", Chapter 6[1], which contains numerous other methods and procedures not in routine use (e.g. artificial substrates and samples for biomass).

Samples collected using this SOP are typically processed following the SOP "Preparing and processing algae samples for taxonomic analysis of diatoms"[2] and analyzed following the SOP "Diatom sample taxonomic identifications"[3]. The benthic algae sampling methods described here are sometimes complemented by a visual assessment using the SOP "Qualitative field characterization of benthic algae in streams"[4].

This SOP covers sample collection and sample handing up to the point of delivery to the DOW Biological Laboratory for storage until they can be processed. Selection of sampling reaches varies by project and is described in project plans, or following the guidelines in the SOP "Sampling reach determination"[5]. Samples are logged into the WQB sample tracking system following the SOP "EDAS data entry and biological data management"[6].

The sources for the collection methods in this SOP are the historical methods used by WQB[1], as well as general methods recommended in the manual "Rapid bioassessment protocols for use in streams and wadeable rivers, Chapter 6"[7].

4b. Summary of Method

Multiple sampling methods may be used, depending on project objectives. A summary or each sample type follows, with guidelines for applicability. The benthic algae field form, Appendix A, can be used to record information regarding the sample, or the sample type can be recorded on the general site visit form of other biological assemblage samples are collected at the same visit.

Qualitative Multihabitat Composite (QualMHC)
This sample method involves collecting small aliquots of attached algae from all major microhabitats in a sampling reach, and adding them to the same container to produce one composite sample. This is the sampling method most used for biological assessments of aquatic life use. The method has the advantage of maximizing the diversity of forms collected, and is flexible enough for applying to wide range of waterbody types. The QualMHC method corresponds to the method historically used by WQB and referred to as the "natural substrates" sample type in previous SOPs and manuals[1]. The method has been given a new name to distinguish it from other natural substrate sampling methods described in this SOP. The QualMHC method can be standardized somewhat by allocating aliquots to a pre-defined set of microhabitats (QualMHC-S).

Qualitative Targeted Habitat (QualTH)
This sample method involves collecting small aliquots of attached algae from only one microhabitat type throughout the sampling reach (e.g. riffle rocks, pool sediments, submerged vegetation). This method has the advantage of minimizing some of the variation in samples due to site-to-site differences in habitat, potentially increasing the ability to observe differences due strictly to water quality. One disadvantage if selecting just one habitat type is that the habitats not sampled may be the ones most sensitive to water quality differences.

Quantitative Targeted Habitat (QuantTH)
This sample method involves collecting small aliquots of attached algae from only one microhabitat, as with QualTH, but the aliquots are sampled from a known area of substrate and the number of aliquots, their areas, and the total sample volume is recorded. Results of the taxonomic analysis and enumeration[3] can be extrapolated to derive an estimate of cells or units per unit area. This method has the advantages and disadvantages of the QualTH, but with the added advantage of producing a measure of areal cell density. Samples collected by this method also could be split for chlorophyll-a or ash free dry mass analyses, following special handling procedures not in this SOP[1].

4c. Definitions and Acronyms

Acronyms:
DEP Department for Environmental Protection
DOW Division of Water
MSDS Material Safety Data Sheets
PPE personal protective equipment
SOP Standard Operating Procedure
WQB Water Quality Branch

4d. Health & Safety

Preservatives for algae samples contain hazardous chemicals. Wading in streams, rivers, and lakes involves physical hazards and hazards associated with unknown pollutants. Do not perform these procedures without fully understanding hazards and appropriate personal protective equipment (PPE). Inattention to safety procedures could result in damage to skin and eyes, bodily injury, and contact with unknown substances and human pathogens.

Material Safety Data Sheets (MSDS) for all chemicals involved in these procedures are kept in the DOW biological laboratory for reference, and relevant MSDS sheets are kept in field vehicles.

All accidents must be reported to the Monitoring Section Supervisor. For life threatening emergencies, call 911 from the cell phone in the field. Disposal of wastes generated from these procedures must follow DEP's chemical hygiene plan or instructions from the Monitoring Section Supervisor.

Field safety guidelines for DOW personnel are detailed in "Methods for Assessing Biological Integrity of Surface Waters in Kentucky"[1], Section 12: Heath and Safety, and in the draft WQB Health and Safety Manual for Field Operations[8]. This manual describes common hazards associated with field sampling in surface waters and personal protective equipment (PPE) appropriate for each hazard. PPE for this tasks described here may include waders, insect repellent, sun block, gloves, and chemical splash goggles (see below). Any hazards encountered during field activities that are not addressed by this SOP or the Field Safety Manual must be reported to the supervisor who will recommend and furnish appropriate PPE, where possible, or recommend that the activity not be performed.

Specific Hazards

Formalin: Samples that must be preserved in the field (as per project specifications) may require handling of formalin. Gloves and chemical splash goggles are required when mixing formalin solutions, when preserving samples, or when handling preserved samples. All use of formalin in the field must be done outdoors in a fully ventilated area.

4e. Cautions

Take special care to label sample jars with all necessary site and sample information.

With quantitative samples, take care to ensure accurate measurements of aliquot areas and volumes, and to carefully record all amounts.

4f. Interferences

Sample degradation: Improper preservation of samples may lead to decomposition of the algal cells and/or continued growth in the sample container. Keep samples on ice during

transport. Preserve in the field if samples will not be delivered to the laboratory within 2 days of collection. Samples that have large clumps of macroalgae or aquatic plants are difficult to preserve effectively. Minimize these materials in samples wherever possible, unless those materials are a target of the sample.

Contamination and cross-contamination: Because algal cells are microscopic, the potential for contamination from dirty equipment and containers is great. Containers must be stored with lids on, in cabinets, or wrapped, to minimize settling of airborne particles. Sampling tools must be thoroughly cleaned before using for the next sample. Keep sample container lids tight.

Insufficient sample amounts: Sufficient material is necessary to process all needed subsamples and to effectively represent the reach. If algal coverage of substrates is very sparse, consider taking more aliquots than planned. There should be visible color to the sample water. While taking aliquots, do not incorporate more water than is necessary.

Excessive sediment in samples: Samples with large amounts of fine sediment make taxonomic identifications difficult. Avoid including excessive amounts of sediments in the sample container. When sampling sediment-associated algae, take care to suction or lift off only the top portion of the sediment surface that contains most of the living algal cells.

4g. Personnel Qualifications / Responsibilities

Procedures should be performed by an Environmental Biologist in the WQB, or personnel with a similar level of general aquatic biology background and experience. DOW personnel performing these procedures will be trained in this SOP by a WQB algae specialist under field conditions.

DOW personnel performing this method will be trained in field safety in accordance with the DOW Health and Safety Manual for Field Operations[8].

Personnel performing these procedures are responsible for fully understanding safety and quality assurance procedures.

4h. Equipment and Supplies

WQB biological assessment site characterization and habitat assessment form ("site visit form")[8], OR benthic algae field sheet (Appendix A)
plastic sample container with tight fitting lid (e.g. 125 mL jar)
permanent marker
compositing jar (if necessary)
metal scraper
disposable transfer pipettes
area delimiter (QuantTH, see Table 1)
graduated cylinder (QuantTH)
cooler with ice

formalin (if required)
gloves and chemical splash goggles (if using formalin)

4i. Step by Step Procedure

4i1. Before beginning

1) Check project study plan for the prescribed sample types to be collected, and verify time period and sampling conditions. Sampling should not be conducted following significant rainfall or flooding unless specifically prescribed by project objectives. For routine biological assessments, allow at least 2 weeks for recolonization after a period of high flow.
2) The benthic algae field form, Appendix A, can be used to record information regarding the sample, or the sample type can be recorded on the general site visit form if other biological assemblage samples are collected at the same visit.
3) Label sample container with permanent marker, or affix a pre-printed label with the following minimum information: station number, waterbody name, location, sample number, date, name of collector, sample type.
4) Rinse sampling tools in site water.

4i2. Sampling process

QualMHC

1) Scan sampling reach for microhabitat types present, see Table 1. Consider different flow velocities and shading when scanning for microhabitats and treat as separate (e.g., if the reach has abundant rocks in fast sunny riffles and in slower shady runs, consider these to be two distinct microhabitats)
2) Take aliquots from all major microhabitats roughly in the proportion they occur in the reach, using the tools and techniques listed in Table 1.
3) Transfer aliquots directly into the sample container, or into an interim compositing jar or bucket, if necessary.
4) If an interim compositing jar is used, mix sample well before filling sample container. For this type of sample, it is not necessary to include the entire amount, as long as a sufficient sample amount is retained (see Interferences, 4f).

Table 1. Microhabitats to be included (if present) in Qualitative Multihabitat Composite samples, guidelines for sampling and quantifying area.

Microhabitat	Description and sampling techniques
Epipelic	Silt and fine sediment habitats usually in depositional areas. Algae may form a thin mat that loosely adheres to the surface. Suction material from the sediment surface using a disposable transfer pipette, or gently lift the algal mat from the surface of the sediment using a spatula or spoon. For

Microhabitat	Description and sampling techniques
	quantitative samples, area can be defined using an inverted petri dish.
Epipsammic	Sand habitats. Collection same as epipelic above.
Epilithic	Rock or other hard surface habitats including manmade structures or rubble, if abundant. Scrape with a sharp tool. For quantitative samples, area can be defined using a shallow ring of PVC pipe or a shape cut out of a piece of rubber.
Epidendric	Woody material (e.g., submerged logs, tree roots). Collection same as epilithic.
Epiphytic	Plant habitats (e.g. aquatic mosses, macrophytes, macroalgae). Scrape, rub, sqeeze, or collect the entire substrate. Quantifying area will vary depending on morphology (e.g., leaf surface area, moss or macroalgae cover.)
Epizooic	Animal habitats including turtle shells, snail shells and other macroinvertebrates. Scrape, collect the entire substrate.

QualMHC-S

The following guidelines for allocating aliquots can be used for a more standardized collection than the more general QualMHC method, if prescribed by the project objectives. If an interim compositing jar is used, be sure that the final volume will fit into the sample container.

<u>High-Gradient Streams</u>

- 5 riffle rocks in a transect
- 2 leaf packs (squeeze)
- 2 aquatic plants or roots
- 2 pool rocks
- 2-3 pieces of wood
- Sediment depositional area
- Sand depositional area
- Any additional microhabitat

<u>Low-Gradient Streams</u>

- 5 leaf packs
- 5 pieces of wood
- 5 aquatic plants or roots
- 2 sediment depositional areas
- 2 sand depositional areas
- Rocks when available
- Any additional microhabitat

QualTH

1) Select the microhabitat to be sampled, according to project plan (e.g. riffle rocks, pool sediments, submerged vegetation)
2) Scan sampling reach for targeted microhabitat
3) Take 10 aliquots (or a different number, depending on project plan) from separate substrate units (e.g. rocks, pieces of wood, areas of sediment deposition) dispersed throughout the sampling reach using the tools and techniques in Table 1. Transfer aliquots directly into the sample container, or into an interim compositing jar or bucket. If an interim compositing jar is used, mix sample well before filling sample container. For this type of sample, it is not necessary to include the entire amount, as long as a sufficient sample amount is retained (see Interferences, 4f).

QuantTH

1) Select the habitat to be sampled, according to project plan (e.g. riffle rocks, pool sediments, submerged vegetation)
2) Scan sampling reach for targeted habitat
3) Take 10 aliquots (or a different number, depending on project plan) from separate substrate units (e.g. rocks, pieces of wood, areas of sediment deposition) dispersed throughout the sampling reach using the tools and techniques in Table 1 and an appropriate area delimiter. Transfer aliquots directly into the sample container, or into an interim compositing jar or bucket. Important: if an interim compositing jar is used, be sure that the entire final volume will fit into the sample container. Alternative: bring each aliquot to the same pre-defined volume in the compositing jar, mix, and transfer a pre-defined portion to the sample container.
4) Record the area of the delimiter and the number of aliquots on the sample label. If the alternative method in step 3 is used, then also record the proportion of each aliquot included in the sample.
5) Measure total sample volume using a graduated cylinder or other pre-marked measurer; record on sample label

.

4i3. Sample handling and records

1) Record the algae sample type (plus total area and total sample volume for QuantTH samples), and any notes on the site visit form or benthic algae field form.
2) Verify that sample labels match form entries; record problems or deviations on site visit form[9]
3) Place samples on ice in a cooler. If samples cannot be delivered to the laboratory in less than 2 days, then preserve with 3-4% buffered formalin by adding 3-4 mL of formalin per of 100ml sample. Use gloves and chemical splash goggles when handling formalin. Record on sample label and site visit form that the sample has been preserved.
4) Upon delivery of samples to the laboratory, complete the algae sample portion of the site visit log in the WQB sample tracking system[5].

5. QUALITY CONTROL AND QUALITY ASSURANCE

QC samples may be prescribed by project plans to document and measure repeatability of the sampling method for producing the final result (e.g. biotic index, rating, metric, density, etc.). Comparisons regarding QC samples will depend on how the sample results are used in the project. For example, if the sampling is for an assessment of aquatic life use support, then the difference in narrative ratings or index scores would be the QC indicator. Typically, a visit to a different sampling reach in the same stream, or a repeat visit to the same reach by a different sampler will serve as a QC sample. The type of QC sample to be performed will be outlined in project plans.

Alternate Reach Samples

The same sampler samples a different sampling reach within the same stream segment. This QC sample type targets the reach to reach variation and the ability of the sampling method to produce a similar result from different reaches in the same segment.

Revisit Samples

A different sampler returns to the same sampling reach at another date the same year or a different year. This type of QC sample targets both temporal variation and variation between samples, or the ability of the method to produce the same result regardless of sampler or time of sampling.

Data Verification Summary

The table below summarizes data quality verification steps involved in this procedure. These steps ensure that the sample is collected appropriately and that the site information is clear and correct. Deviations from established methods, or use of a different method than the one prescribed by the study plan must be noted and explained. These notes must be entered into the sample tracking system when the samples are logged in. In addition to verification steps, all cautions and interferences listed in sections 4e and 4f of this SOP should be observed in order to ensure quality samples. Data verification steps that follow sample and visit login are described in the SOP "EDAS data entry and biological data management"[6].

When	Inputs	Element Verified	Verification Records
before beginning (4i1-1)	study plan	sample type and conditions	record problems or deviations on site visit form[9]
while completing visit form (4i3-1)	sample	sample type	record problems or deviations on site visit form[9]
while completing visit form (4i3-2)	sample, site visit form[9]	sample info on label and visit form match	none, resolve before leaving site

6. REFERENCES

1. Kentucky Division of Water, 2008. Methods for Assessing Biological Integrity of Surface Waters in Kentucky, Revision 3.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

2. Kentucky Division of Water, 2009. Preparing and processing algae samples for taxonomic analysis of diatoms, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

3. Kentucky Division of Water, 2009. Diatom sample taxonomic identifications, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

4. Kentucky Division of Water, 2009. Qualitative field characterization of benthic algae in streams, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

5. Kentucky Division of Water, 2009. Sampling reach determination, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

6. Kentucky Division of Water, 2009. EDAS data entry and biological data management, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

7. Barbour, M.T., J. Gerritsen, B.D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

8. Kentucky Division of Water, 2007. DOW Health and Safety Manual, in draft. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

9. Kentucky Division of Water, 2009. Site characterization and habitat assessment in wadeable streams, Revision 1.0. Energy and Environment Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

7. ATTACHMENTS/CHECKLISTS AND APPENDICES

Appendix A: benthic algae field sheet

APPENDIX A: BENTHIC ALGAE FIELD SHEET (NEXT PAGE)

BENTHIC ALGAE FIELD DATA SHEET

DOW Station ID Number:	**Stream Name**:	**Location**:
Collection Date:	**Time**:	**County**:
River Basin:	**DOW Program**:	**Name of Investigator**(s):

Sampling visit associated information (circle): Habitat form Multiprobe Water Sample Fish Macroinvertebrates

Sample type(s) **Number of samples _____**	QualMHC (all habitats) QualMHC-S: low gradient high gradient QualTH: riffle rocks sediments other (_____) QuantTH (habitat _____; total area_____cm^2; total sample vol _____mL) Other: _____
Macrohabitats Sampled:	Riffles Runs Pools Margins/Backwaters
Microhabitats Sampled (composite samples):	Silt Sand Rock Wood Roots Moss Plants Leaves Animal Shells Artificial substrate
Microalgae Thickness (sampled substrate):	Thickness: **0** absent **1** "slimy" **2** "visible" **3** 1-5 mm **4** 5-20 mm **5** >20 mm

Benthic Algae Visual Assessment

Macroalgae taxa present:_____

Macroalgae sampled for ID? Yes No

Macroalgae cover: approximate % of suitable substrate covered

	absent	sparse < 5%	Common 5 to 25%	Abundant 25-50%	Extensive >50%
Green Filaments					
Floating Mats or Scums					
Bluegreen Mats					
Diatom Mats					
Red Algae					

Suspended algae: not evident low to moderate water column appears green

Notes: