ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-64
Rev. 0 (10/00)

FIELD SAMPLING PROCEDURES FOR THE NEW JERSEY ALGAE INDICATORS PROJECT

Prepared by: Donald Charles, Diane Winter and Michael Hoffman

Approved by: _____ _____
Robin S. Davis Date
Quality Assurance Manager

Field Sampling Procedures for the New Jersey Algae Indicators Project

1. PURPOSE:

1.1. The Academy of Natural Sciences (ANS) located in Philadelphia, PA has entered into a contract with the New Jersey Department of Environmental Protection (NJ DEP) for the purpose of developing algal indicators of stream and river eutrophication. These indicators will be used to assess relationships between extant water quality criteria (e.g., phosphorus and nitrogen concentrations) and overt signs of eutrophication. They will be applied in a regulatory context as secondary criteria for identifying nutrient impairment. These indicators will be based on an understanding of algal dynamics in New Jersey streams, and will be able to distinguish between situations where nutrient concentrations are high due to natural environmental conditions and those that result from anthropogenic influences. Protocols are needed that describe procedures for sample collection and processing, analysis and presentation of data, and interpretation of results. Research performed by the ANS includes analysis of algal samples, interpretation of data, synthesis of results and formation of a protocol for use in future sampling endeavors

1.2. The study sites correspond to New Jersey Department of Environmental Protection (NJDEP) sites monitored for water quality, benthic invertebrates and/or fish. Three types of algal and water samples will be collected. This protocol was developed specifically for this project. The Algal Biomass Samples (ABS) are quantitative and will be analyzed for soft algae, diatoms, chlorophyll *a*, and Ash Free Dry Mass (AFDM). The Diatom Composite Samples (DCS) are qualitative, representing diatoms on rocks with no filamentous algae that are located in portions of sampling sites with faster flowing current. These samples will analyzed for diatoms alone. The Cover Type Samples (CTS) are collected to identify the dominant species of benthic algae at each study site.

2. SCOPE:

2.1. While this subsampling procedure is applicable mainly to NJAIP periphyton sampling sites, it can be followed for all algal sampling where similar objectives are to be met.

2.2. This procedure applies to all personnel responsible for collecting algal samples in the field.

3. REFERENCES:

3.1. 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. US Environmental Protection Agency; Office of Water; Washington, D.C.

3.2. Porter, S.D., T.F. Cuffney, M.E. Gurtz, M.R. Meador. 1993. Methods for collecting algal samples as part of the National Water Quality Assessment Program. Raleigh, North Carolina.

4. APPARATUS/EQUIPMENT:

4.1. Large plastic pans.

4.2. Assorted brushes for removing algae from rocks.

4.3. Nalgene bottles; 20 ml, 125 ml, and 250 ml.

4.4. Squirt bottles.

4.5. Water proof paper and pens, Sharpies Tm.

4.6. Covered clipboard.

4.7. Camera and film.

4.8. Waders, felt-bottomed boots, rain jackets.

4.9. Insect repellent, with DEET for ticks.

4.10. Scissors and scalpels.

4.11. Formaldehyde, plastic gloves, protective eye-wear, plastic/glass pipettes.

4.12. Viewing buckets/aqua-scopes.

4.13. Waterproof measuring tape (50-100 m).

4.14. Pre-cleaned 125-ml bottles (HDPE), 25-mm glass filters, Gelman 25-mm pre-cleaned filter holder, 60-ml pre-cleaned plastic syringes (Beckman), distilled water, dilute HCl, forceps.

4.15. Heavy duty aluminum foil, paper towels, Kimwipes.

4.16. Coolers and ice.

4.17. Strapping, clear and labeling tape.

4.18. ANS field sampling forms (attached).

4.19. Densiometer

5. SAFETY PRECAUTIONS:

5.1 As samples for analysis of algal species composition are preserved in formalin (2-10%), protective eye-wear and plastic gloves should be worn while handling.

6. PROCEDURES:

6.1 Initial Site Characterization

6.1.1 Location and Assessment of Site to Determine Sampling Area.

6.1.1.1 The NJDEP monitoring sites are defined as the intersection of a road and the river or stream to be sampled. Most sampling done by the NJDEP is on the upstream side of the bridge to minimize effect of the bridge and of automobile use. Above the bridge is the preferred location for algal sampling also; however, the downstream side may be more acceptable if it is considered more representative of river habitat within the region.

6.1.1.2 Identify section of river to be sampled ("sampling reach" in NAWQA terminology) and division of sampling area into three sections.

6.1.1.2.1 Ideally the sampling area should contain three riffles and three pools; if these are not present at the site, collect samples from three equally sized sections.

6.1.1.2.2 If the above criterion is not appropriate for the site, length of reach should be defined as 10 to 20 channel widths.

6.1.1.2.3 Regardless of method used, "minimum and maximum acceptable ranges are 150 to 500 m for wadeable sites and 500 to 1,000 m for nonwadeable sites" (NAWQA field sampling protocols, Porter et al. 1993).

6.1.1.2.4 Border of reach should be measured as distance up or downstream from bridge. Length of reach should be measured and recorded on the Site Description sheet.

6.1.2 Fill out heading and initial data on Site Description sheet.

6.1.2.1 Site location, data, persons collecting, etc.

6.1.2.2 Description of location of site and breakdown of reach into three sections.

6.1.2.2.1 Indicate if site is upstream or downstream from bridge; if downstream, provide rationale for this choice.

6.1.2.2.2 Draw simple sketch of sampling reach and sections, indicating major habitat characteristics and unique structures or criteria that could be used to relocate the site.

6.1.2.2.3 If staff gage is present, record water level height.

6.1.2.3 Continue making notes throughout sampling period.

6.1.3 Make measurements of river characteristics, to the extent that is practical (this may not be possible for sites with rapid flow or which are very deep).

6.1.3.1 Measure width and maximum depth (in m).

6.1.3.2 Estimate velocity of stream or river; fast, medium or slow.

6.1.3.3 Measure canopy density with spherical densiometer; take measurements midstream at the center of each of the three sampling reach sections, in areas from which rocks will be/were collected. In addition to densiometer readings, categorize sampling reach as open, semi-shaded or heavily shaded.

6.1.4 Take pictures of the habitat and/or the area sampled.

6.1.4.1 Both upstream and down.

6.1.4.2 Features relevant to local habitat quality (active erosion sites; local residential or commercial development).

6.1.4.3 Typical rocks in streams with typical algal growth; whole areas of stream and individual rocks.

6.1.4.4 Record pictures taken on Photograph form. If digital camera used, record image filename.

6.2 Algal Sampling Procedures

6.2.1 Collect composite samples for algal biomass (chlorophyll *a*, AFDM) and algal species composition.

6.2.1.1 Select at least three rocks from each of the three sections of the sampling reach. There will be a total of at least nine rocks for each sampling reach. Each set of rocks will constitute one composite sample, there will be three composite samples per sampling reach (one sample per bottle).

6.2.1.2 Select rocks from the main part of river, avoiding areas very near the shore, if possible. Avoid heavily shaded areas; select unshaded sites if possible. Rocks should be 5 to 25 cm in greatest dimension and generally as representative of other rocks as possible; select from different sections in the sampling reach. If there are no larger rocks, select 5-10 smaller rocks. If there are no rocks at all, or very few, especially if there are none upstream either, do not sample the site. A good strategy is to select six rocks, randomly, then keep the three that are most representative.

6.2.1.3 Place the three selected rocks in a shallow white pan. Prepare a 3 x 5 card, or similar label with site name, date and river section . Use thick dark marker. Place label near pan and take picture of rocks in pan.

6.2.1.4 Put the selected rocks in a deep-walled, plastic pan. Scrape all algae from the rocks using nylon brushes, scalpel, knife, or spoon. Use a fine spray from a squirt bottle (filled with clean river water) to wash algae from rocks (remember that total sample volume should fit in one 250-ml Nalgene bottle). Use scissors to cut non-diatom filamentous algae into pieces no longer than 0.25 to 0.5 cm. Remove small rocks from the sample. Pour the sample from the pan into labeled, tared 250-ml sample bottles. The label should be written on tape and include site name, date, reach section, number of rocks scraped, and collectors' initials.

6.2.1.5 Store samples in cooler on wet ice.

6.2.1.6 Note on field data sheet the number of samples collected and number of rocks represented.

6.2.1.7 Trace the outline of each rock on waterproof paper. More than one rock can be outlined per page; number each rock outline sequentially. Label each page with site name, date, number of rocks collected from each site, and initials of person making the outline.

6.2.1.8 Wrap aluminum foil around the surface of each rock, covering the area that was scraped to remove algae. Press foil tightly to rocks. Either trim bottom edge with scissors or fold excess foil upwards. Remove foil from the rock and make radial cuts in foil to allow the foil to be flattened. Either in the field, in motel, or back at the lab, place each foil on paper (waterproof if in field) and trace the outline. Label each page with site name, date, number of rocks collected from each site, and initials of the person making outline.

6.2.1.9 Repeat the procedure for the other two sampling sections in the reach.

6.2.2 Collect qualitative samples for taxonomic analysis of diatom assemblages.

6.2.2.1 Collect an additional five or more rocks from each of the three sampling reach sections.

6.2.2.2 Rocks should be 5 to 25 cm in greatest dimension and generally as representative of other rocks as possible. Select them from different parts or areas in the reach section, in mid-river areas of rapidly moving water. Select rocks with a film of diatom-like growth. Avoid, to the extent possible, rocks with non-diatom filamentous algal growth or rocks covered with layers of silt and clay.

6.2.2.3 Use brushes and a squirt bottle to remove algal growth from about 4 to 6 cm^2 of surface of each rock. Make sure tools used to scrape the rocks are clean and free of diatoms from previous sampling events. Collect algae in a plastic pan. Brush thoroughly to get all closely adhering diatoms from the rock surface. Avoid including algae from outside the brushed area. Avoid including filaments of non-diatom algae. Rinse rock areas with a small amount of water from a squirt bottle.

6.2.2.4 Pour composite sample from each sampling reach section into a separate bottle; there will be three 125-ml bottles from each sampling reach. Add buffered formalin as preservative; the final concentration should constitute 3 to 5% of the total sample (NAWQA Field Protocol); closer to 3% if the sample is mostly diatoms. Label the bottle with sampling reach and section, date, collectors' initials, and number of rocks.

6.3 Substrate Characterization.

6.3.1 Estimate percent of physical substrate categories listed on Inorganic Substrate Components sheet.

6.3.1.1 For each section of the sampling reach estimate the percent of the bottom surface covered by silt/clay, sand, gravel, cobble and boulder.

6.3.1.2 Estimates should be an average for each section of the reach and can be based on observation from shore, use of viewing bucket, or other approach.

6.3.1.3 Estimates should be made to the nearest 5-10 %. Describe the methods used.

6.3.1.4 Make notes on factors affecting the accuracy and variability of the numbers, and factors affecting them (e.g., bottom not visible). These numbers will used as estimates of the percent of bottom covered by substrate particles > 2 cm in diameter.

6.3.2 Estimate the amount of bottom covered by various algal types - EPA Periphyton Rapid Bioassessment. (Use the procedure most appropriate and practical for the site. The following is a method that should apply to most streams. This technique can be modified to meet local conditions.)

6.3.2.1 Viewing bucket method: this works for most streams except very large or small ones. The bottom must be visible. The goal of this procedure is to estimate the percent of the bottom composed of rocks > 2 cm in diameter that are covered by microalgae and macroalgae (e.g., filamentous algae), and to estimate the thickness of that algae.

6.3.2.1.1 Make a series of estimates of bottom conditions by looking through a bucket with a clear plastic bottom. Space observations at about 1-m intervals across the river. Vary the width accordingly if this would lead to fewer than 15 or more than 20 observations.

6.3.2.1.2 At each observation point, estimate to nearest 5% the percent of bottom covered by algal growth categories described in the EPA Rapid Bioassessment document (page 6-18, Barbour et al. 1999). Define new micro- and macro-algal types as they occur. Generally, there should be no more than 4-5 algal types per sampling reach.

6.3.2.1.3 Collect algal samples of distinctive macroalgal types found in the main channel. Generally there will be no more than 4 or 5 dominant distinctive algal types.

6.3.2.2 Modifications to viewing bucket method.

6.3.2.2.1 If the water is too cloudy to see the bottom, pick up individual rocks along transects and make observations.

6.3.2.2.2 If the river is narrow and the bottom is clearly visible, estimates can be made from shore without using viewing bucket.

6.3.2.2.3 If the river is too deep and fast flowing to wade across, make estimates based on the area near shore where rocks can be safely removed from the bottom.

6.4 Water Chemistry Sampling Procedures for Nutrients. (See Attachment 3 for ANS Procedure for Syringe Water Sampling and Filtration for the Collection of Filtered Nutrient Samples and Unfiltered Nutrient Samples.)

6.4.1 Water samples will be collected initially upon arrival at each site, before other collection activities are performed.

6.4.2 At the beginning of each day, two field blanks will be prepared. These will be prepared in an identical manner to a real sample, but instead of using water from a river or stream, distilled water will be substituted. There will be one filtered and one unfiltered field blank.

6.4.3 One site each day will be sampled twice, for quality control purposes.

6.4.4 Actual sampling method:

6.4.4.1 Place paper towels on top of the work area.

6.4.4.2 Take out two 125-ml, pre-cleaned bottles and label appropriately for each site (or field blank).

6.4.4.3 Prepare a clean 60-ml syringe, filter holder and 25-mm glass filter.

6.4.4.3.1 Rinse the inside of the syringe, the syringe plunger and filter holder quickly with dilute acid and then thoroughly with distilled water.

6.4.4.3.2 Place the filter into the holder with forceps or tweezers.

6.4.4.4 Fill the syringe with clean stream water (collect sample water from below the surface of the water) or distilled water.

6.4.4.5 Place the plunger back into the syringe and rinse the filter with ~ 5 ml of water. Rinse the 125-ml bottle three times with 5 ml of filtrate, or distilled water. Tighten the cap on the bottle while rinsing to also rinse the cap. Fill

a prepared bottle with ~ 100 ml of filtered water, re-filling the syringe as needed.

6.4.4.6 For the unfiltered sample, rinse the 125-ml bottle and cap three times with ~5 ml of stream water or distilled water prior to filling with sample. Fill the second bottle with unfiltered water directly from the stream (or unfiltered distilled water).

6.4.4.7 Make sure both bottles are labeled correctly and place directly in the cooler with wet ice.

6.4.5 Change the filter after each sample is taken (a sample consists of one 125-ml bottle), and also after the field blanks.

6.5 Preparing Samples for Shipment.

6.5.1 Make sure all bottles are tightly sealed and properly labeled.

6.5.2 Place water chemistry bottles in ziploc bags, one site per bag. Place these in upright position in the bottom of the cooler. It is very important that these do not tip over during transit to ANS.

6.5.3 Place algal sample bottles in the cooler.

6.5.4 Double bag ice in ziploc bags to prevent leakage, and place around and on top of bottles to be shipped.

6.5.5 Fill out Sample Custody Forms before leaving each site. Place forms in ziploc bags and tape to the lid of the cooler. Make a separate form for each type of sample (ABS, DCS, CTS, Nutrient), giving a detailed description of each sample and what should be done with each upon receipt in the lab at ANS.

6.5.6 Close and seal cooler with strapping tape. Affix FedEx or other label to the lid of the cooler.

6.5.7 Ship samples for overnight delivery to arrive at the lab the next morning. If samples are not shipped, bring directly to the lab the day on which they are sampled.

7. QUALITY ASSURANCE/QUALITY CONTROL:

7.1 These procedures were developed by the Patrick Center for Environmental Research at The Academy of Natural Sciences. They are a combination of several different source references, which have been adapted to work with the current project.

7.2 Because algae are microscopic, the possibility of contamination of samples is great. Brushes used to remove algae from the rocks and the pans used to collect them should be cleaned as thoroughly as possible between each use.

7.3 Quantitative samples need to be mixed well when sampling or during the subsampling (possibly blended), to avoid clumps caused by natural growth forms (colonies, filaments, etc.).

7.4 All deviations from this protocol must be noted in field and/or laboratory notebooks at the time of the deviation or, at the time deviations are realized. If the deviation is such that the quality or integrity of the study is affected, the Lab Manager must be informed immediately.

7.5 Minor modifications of project protocols may be necessary. Minor changes are to be noted in the margin, initialed and dated. This may be done by the Principal Investigator, the Lab Manager or the staff member responsible for performing the procedures outlined in the protocol. All such notes must be also entered into the master copy and a copy sent to the QAU.

7.6 In the case of a formal revision, the protocol will carry the same procedure number but will be given a revision number and revision date. Formal revisions must be reviewed by the Principal Investigator or the Lab Manager and must be approved by the manager of the QAU.

8. REFERENCES:

8.1. Porter, S.D., Cuffney, T.F., Gurtz, M.E., Meador, M.R. 1993. Methods for collecting algal samples as part of the National Water Quality Assessment Program. Raleigh, North Carolina.
8.2. Syringe Water Sampling and Filtration for the Collection of Filtered Nutrient Samples and Unfiltered Nutrient Samples. Procedure No. P-16-119, Rev. 0.

9. ATTACHMENT:

9.1. Examples of Sample Data sheets used; Site Description, Site Drawing, Section Description, Photographic record, Inorganic Substrate Components, Rapid Periphyton Survey Field Sheets (2 types).

Attachment 1
NJAIP SAMPLE DATA SHEETS