

New Jersey Periphyton Bioassessment PROTOCOL MANUAL

(Standard Operating Procedures: Field, Lab, Analysis)

Trophic Diatom Inference Models and Index Development for New Jersey Wadeable Streams

**Submitted to
Thomas Belton, Project Manager**

**New Jersey Department of Environmental Protection
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**Understanding the Relationship Between Natural Conditions and Loadings on
Eutrophication: Algae Indicators of Eutrophication for New Jersey Streams.**

Protocol Manual

Submitted to:

New Jersey Department of Environmental Protection
Division of Science, Research and Technology

Submitted by:

The Academy of Natural Sciences
The Patrick Center for Environmental Research
Phycology Section

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ACRONYMS

ANSP	The Academy of Natural Sciences of Philadelphia
APHA	American Public Health Association
ASR	Analytical Services Request
AWWA	American Water Works Association
BIO-TDB	Biological Transitional Database
CAR	Corrective Action Report
DCF	Dilution/Concentration Factor
DHDB	Diatom Herbarium Database
DIC	Differential Interference Contrast
DTH	Depositional Targeted Habitat
DW	Distilled Water
EPA	Environmental Protection Agency
FTP	File Transfer Protocol
ID	Identification
MSDS	Material Safety Data Sheet
NADED	North American Diatom Ecological Database
NAWQA	National Water-Quality Assessment
PC	Personal Computer
PCER	Patrick Center for Environmental Research
PIMS	Phycology Information Management System
PP	Phytoplankton
PPE	Personal Protective Equipment
PSI	Pounds per Square Inch
QA	Quality Assurance
QAM	Quality Assurance Manager
QAU	Quality Assurance Unit
QC	Quality Control
QMH	Qualitative Multihabitat
RO	Reverse Osmosis
RTH	Richest Targeted Habitat
SOP	Standard Operating Procedure
USGS	United States Geological Survey
WEF	Water Environment Federation
WPCF	Water Pollution Control Federation

INTRODUCTION

GENERAL SCOPE

The Academy of Natural Sciences (ANSP) located in Philadelphia, Pennsylvania has entered into a Cooperative Agreement (Co-Op) with the New Jersey Department of Environmental Protection (NJ DEP) for the purpose of development of 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 are based on an understanding of algal dynamics in New Jersey streams, and 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 ANSP includes analysis of algal samples and interpretation of data, synthesis of results and formation of a protocol for use in future sampling endeavors.

This study was initiated in July 2000. Years 1 and 2 of this project were limited to development of algae indicators in the Piedmont physiographic province in New Jersey (Ponader and Charles, 2003). During the third year the study was expanded to include sites in the Highlands and the Ridge and Valley physiographic provinces. Data from sites studied during all three years were used to successfully develop and test indicator metrics for the northern part of the State (Ponader et al., submitted). The fourth and fifth year of the study extended the development of indicators to the Inner and Outer Coastal Plain. Data from sites studied during year four were used to develop and test additional indicator metrics (Ponader and Charles, 2004). During the fifth year, samples were taken in the Outer Coastal Plain to provide additional data that will allow development of a diatom index to be used in the Coastal Plain physiographic province of southern New Jersey (research ongoing).

PURPOSE:

This Protocol Manual has been prepared for the New Jersey Department of Environmental Protection (DEP) as part of the Co-Op to describe sample collecting, sample handling and analytical protocols. The main goal of this Manual is to provide the NJ DEP with the necessary protocols to allow them to continue this work within the NJ DEP. These protocols describe procedures that were used in the field and for laboratory analysis of algae samples collected for the project “Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algae Indicators of Eutrophication for New Jersey Streams” (2000-2005) by the Patrick Center for Environmental Research (PCER) at The Academy of Natural Sciences in Philadelphia (ANSP). This Protocol Manual is different from the Quality Assurance Plan (QAP), which has been prepared as part of the Co-Op to describe sample collecting, sample handling and analytical protocols, reporting procedures, and Quality Assurance /Quality Control (QA/QC) activities which were used for work performed under the Co-Op (PCER, 2000). The QAP covers all steps under the contract, from

receipt and log-in of samples at ANSP, to final transmission of data to NJ DEP and only applied to the Co-Op. In contrast this Protocol Manual only focuses on a more detailed description the field and lab procedures.

Individual protocols were written and reviewed by staff who perform the analyses and are in the format specified by the PCER Quality Assurance Unit (QAU). This format requires that each protocol be understandable and usable by itself (independently), in conjunction with listed references. Most protocols were developed specifically for sampling and analysis of this Co-Op. Some additional protocols that are integrated into this Protocol Manual were originally written for procedures followed when handling National Water Quality Assessment Program (NAWQA) sample analysis. They contain the phrase “USGS NAWQA Program” in their title. All protocols have been reviewed and approved by the ANSP Quality Assurance Manager (QAM). All NAWQA protocols are also available at <http://diatom.acnatsci.org/nawqa/protocols.asp>. Most protocols have been in use since 2000, and some only since 2003 when ANSP began analyzing samples from the NJ Inner and Outer Coastal Plain.

The protocols apply to the collection and analysis of the four following types of algal samples:

1) Algal Biomass Samples (ABS) are quantitative periphyton samples are collected from rocks (5-25 cm in greatest dimension). In rivers with predominantly sand/silt sediments(Coastal Plain), they are collected from either artificial substrate (Diatometers) or directly from the epipsammic/epipellic substrate using the protocols provided). Each sample is a composite sample collected from a defined sampling reach. These samples will be analyzed for chlorophyll *a*, and Ash Free Dry Mass (AFDM). Subsets of these same samples will be used to measure diatom and soft algal abundance, species composition, and community structure.

2) Diatom Taxonomy Samples (DTS) are qualitative samples taken from rocks (5-25cm in greatest dimension) representative of non-filamentous diatom growth for the stream habitat. These rocks will be chosen from those located in the region of the stream with highest water velocity. In rivers with predominantly sand/silt sediments(Coastal Plain), they are collected from either artificial substrate (Diatometers) or directly from the epipsammic/epipellic substrate using the protocols provided). Each sample is a composite sample collected from a defined sampling reach. The DTS will be analyzed for diatom abundance, species composition, and community structure.

3) Periphyton Rapid Bioassessment Samples (PRBS) will be selected as those representative of the most dominant algal form in area of the stream being studied. These will be identified and categorized for all algal types present.

4) The Water Chemistry samples (WCS) are taken for the collection of filtered water for dissolved nutrients and organic carbon and unfiltered samples for various parameters.

Because of differences in predominant substrates in the rivers of the different ecoregions, two different protocols were developed for the collection of all types of samples from two different types of substrates a) the most common hard substrate, usually rocks, or b) from sand/silt substrate as the predominant substrate.

REFERENCES:

Patrick Center for Environmental Research. 2000. Quality Assurance Project Plan for the project “Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams”, Academy of Natural Sciences, Philadelphia, PA. 21 Pages.

Ponader, K., and D. Charles. 2003. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams - Final Report Year 2. Report No. 03-04. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA. 74 Pages. Also available at:
<http://www.state.nj.us/dep/dsr/wq/wq.htm>

Ponader, K., and D. Charles. 2004. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams - Final Report Year 4. Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA. 46 Pages.

Ponader, K. C., D. F. Charles and T. J. Belton. (in review). Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecological Indicators. Submitted May, 2005 (in review).

1. Field protocols

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

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.
- 4.3. Squirt bottles.
- 4.5. Water proof paper and pens, Sharpies[™].
- 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. Densimeter

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 cm² 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 be 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.

Procedure No. P-13-64

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.

REFERENCE:

Procedure No. P-13-64

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.

ATTACHMENTS:

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)

Procedure No. P-13-64

Attachment 1
NJAIP SAMPLE DATA SHEETS

Site Description

Site Name: _____ Site #: AN- _____

Date: _____ Time arrived: _____ Time finished: _____

Collectors: _____

Weather/Temperature: _____

Velocity of stream (m/sec): _____; slow moderate fast

Average Width (m): _____ Temp (°C): _____ pH: _____

Reach Length (m): _____ Conductivity (µS): _____ water color _____

Site Description _____

Miscellaneous Notes:

Procedure No. P-13-64

Site Name: _____ Site #: AN- _____ Date: _____

Site Drawing:

Site Name: _____ Site #: AN- _____ Date: _____

Section 1:

Length of Section (m): _____ Velocity (m/s): _____; slow moderate fast

Densiometer:

of openings counted: _____

of openings *1.04: _____

Algae Biomass/Chlorophyll a/soft algae (quantitative):

of Rocks Sampled: _____

*** outlines of rocks on tracing sheet attached

Diatoms(qualitative):

of Rocks Sampled: _____

Average Diameter of rocks: _____

Section 2:

Length of Section (m): _____ Velocity (m/s): _____; slow moderate fast

Densiometer:

of openings counted: _____

of openings *1.04: _____

Algae Biomass/Chlorophyll a/soft algae (quantitative):

of Rocks Sampled: _____

*** outlines of rocks on tracing sheet attached

Diatoms(qualitative):

of Rocks Sampled: _____

Average Diameter of rocks: _____

Section 3:

Length of Section (m): _____ Velocity (m/s): _____; slow moderate fast

Densiometer:

of openings counted: _____

of openings *1.04: _____

Algae Biomass/Chlorophyll a/soft algae (quantitative):

of Rocks Sampled: _____

*** outlines of rocks on tracing sheet attached

Diatoms(qualitative):

of Rocks Sampled: _____

Average Diameter of rocks: _____

Photographs:

Site Name:_____ **Site #: AN-**_____ **Date:** _____

Picture # _____

RAPID PERIPHYTON SURVEY FIELD SHEET

Stream Name: _____

Site #: AN- _____

Date: _____

Transect width: _____

Collectors Initials: _____

Microalgae 1: _____

Microalgae 2: _____

Microalgae 3: _____

Macroalgae 1: _____

Macroalgae 2: _____

Macroalgae 3: _____

<i>Thickness Rankings (microalgae only)</i>
0 - substrate rough with no visual evidence of microalgae
0.5 - substrate slimy, but no visual accumulation
1 - a thin layer of microalgae
2 - accumulation of microalgal layer from 0.5 - 1 mm
3 - accumulation of microalgal layer from 1 mm to 5 mm
4 - accumulation of microalgal layer from 5 mm to 2 cm
5 - accumulation of microalgal layer greater than 2 cm

Macroalgae 1 Max. Length: _____

Macroalgae 2 Max. Length: _____

Macroalgae 3 Max. Length: _____

Section # / Transect	Macro algae #1: %Cover	Thick ness Rank	Macro algae #2: % Cover	Thick ness Rank	Macro algae #3: %Cover	Thick ness Rank	Micro algae #1: %Cover	Thick ness Rank	Micro algae #2: %Cover	Thick ness Rank	Micro algae #3: %Cover	Thick ness Rank

Comments: _____

Inorganic Substrate Components

Site Name: _____ Site #: AN-_____

Date: _____ Assessed By: _____

Substrate Types	Diameter	% Composition in Sampling Area			
		Section 1	Section 2	Section 3	Total Reach
Bedrock					
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"-10")				
Gravel	2-64 mm (0.1" - 2.5")				
Sand	0.06-2 mm (gritty)				
Silt	0.004-0.06 mm				
Clay	< 0.004 mm (slick)				

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-119
Rev. 0 (10/00)

**SYRINGE WATER SAMPLING AND FILTRATION FOR THE
COLLECTION OF FILTERED NUTRIENT SAMPLES AND
UNFILTERED NUTRIENT SAMPLES**

Prepared By: David Velinsky

Syringe Water Sampling and Filtration for the Collection of Filtered and Unfiltered Nutrient Samples

1. PURPOSE:

The purpose of this procedure is the preparation of water samples for the collection of filtered water for dissolved nutrients and organic carbon and unfiltered samples for various parameters. Other chemical constituents can be collected with modifications of this procedure.

2. SCOPE:

- 2.1. This field method is applicable to most types of less turbid waters. Various types of filters can be used dependent on the type of filtered constituents that are sought. Caution must be noted due to the type of syringe and the plastic components. The typical Beckman all plastic syringe has a black rubber plunger which is not suitable for trace metals and organic contaminants.

3. APPARATUS/EQUIPMENT:

- 3.1. 125-ml HDPE pre-cleaned bottles (See Cleaning SOP: In brief, rinse bottle with micro soap, deionized water (DI) water rinse, soak in 0.5N HCl for two days, rinse with copious amounts of DI water).
- 3.2. 60-ml pre-cleaned plastic syringe (Beckman). Can use all glass syringe for organic compounds.
- 3.3. Gelman 25-mm pre-cleaned filter holder (note: rinse unit and metal screen with a little acid and quickly rinse with plenty of DIW).
- 3.4. Two metal tweezers.
- 3.5. 25-mm Whatman GFFs (note: pre-combusted (pre-heated at 450 °C for 2 hr) for dissolved organic carbon (DOC)). Other filters can be used dependent on chemical parameter sought.
- 3.6. 30 or 60 ml pre-cleaned glass or Teflon bottles (DOC).
- 3.7. Large quantities of high quality deionized water (DIW) with squeeze bottle. Volume dependent on the amount samples being collected. The DI water must have a resistance of > 14 megaohms; e.g., double deionized water, distilled deionized water.

- 3.8. Large quantities of dilute HCL (0.5N HCL) and squeeze bottle for rinse acid. Volume dependent on the amount samples being collected.
- 3.9. Sharpie marker pens.
- 3.10. Roll of marking tape.
- 3.11. Scissors.
- 3.12. Waste bottle for acid rinses, garbage bags, and quart and gallon size plastic Zip-loc bags.
- 3.13. Cooler for equipment: Do not put food in cooler.
- 3.14. Cooler for dry ice to freeze samples (Samples can be kept cold, 4°C, during the day but it is best to freeze the samples as soon as possible). Do not put food in cooler.
- 3.15. Pre-cleaned plastic or glass cup (pitcher) to collect water if needed.
- 3.16. Kimwipes and paper towels (one or two rolls).

4. SAMPLING AND FILTRATION:

4.1. Unfiltered Water Samples

4.1.1. Field Blanks

- 4.1.1.1. Rinse bottle, syringe or pitcher three times with a little dilute HCl and copious amounts of DIW.
- 4.1.1.2. Rinse equipment at least three times with DIW and fill for equipment rinsate blank.
- 4.1.1.3. Rinse sample bottle three times with 5 ml of water and discard water.
- 4.1.1.4. Pour water from pitcher or push from syringe water into sample bottle and label bottle as equipment rinsate blank (i.e., Field Blank).

4.1.2. Sample Collection

- 4.1.2.1. Rinse collection pitcher, syringe or bottle three times with sample water.
- 4.1.2.2. Pour or push water into sample bottle, rinsing the bottle three times with 5 ml of water prior to filling. Fill bottle 3/4 full of water and cap tightly. Wrap parafilm around top to ensure seal, if needed.
- 4.1.2.3. Rinse sampling/syringe equipment with a little HCL and copious amounts of DIW between stations. Place equipment in “clean” plastic bag for transport.

- 4.1.2.4. **USE A SIMPLE FIELD IDENTIFICATION CODE SYSTEM TO LABEL BOTTLE.** Also, place the letter U on bottle to indicate unfiltered samples. For example, for nutrients use UNUTS, for organic carbon use UOC etc. (Example: Station ID - Site ID - Date UNUTS)

4.2. Filtered Water Samples

4.2.1. Field Blanks

- 4.2.1.1. Beginning of day, rinse equipment (syringe, pitcher, filter holder, etc) with a little acid and lots DIW, if they were used the previous day. If they are clean from the lab this is not necessary. Place equipment in clean or plastic bags for transport. Note: Use paper towels on top of cooler or appropriate surface as a clean "bench top" for filtration.
- 4.2.1.2. Place filter into filter holder using tweezers and wet filter with a little DI water. NOTE: matted side of filter towards metal screen. Carefully place the O-ring into the filter holder and screw top back on; finger tight only.
- 4.2.1.3. Do field blank first: Use DI water as "sample" water. Use all cleaned collection equipment.
- 3.2.1.4. Collect sampled water from below the surface of the water: Rinse cup or pitcher three times with water and then collect water. Alternatively, the water can be sucked directly into the syringe if possible from small stream or creek. Take multiple syringes at one time.
- 3.2.1.5. Rinse pre-cleaned syringe with sample water three times. Suck up about 10 ml and rinse all 60 ml volume. Discard all rinses, then fill syringe with water to 60-ml mark. Rinse filter with a little water (approx. 5 ml), and filter a little water (approx. 5 ml) into collection bottle. Rinse collection bottle three times with 5 ml filtrate each time. Now begin filling bottles. NOTE: If filter becomes clogged because of too much particulate matter (i.e., high turbidity), place new filter in holder, rinse filter and discard rinsate, and proceed. If syringe is empty and more water is needed, remove filter holder, fill syringe, rinse filter with sample water, and continue to fill bottles. **FILL COLLECTION BOTTLE ONLY 3/4 FULL WITH FILTRATE.**
- 3.2.1.6. Cap bottle tightly and wrap top with a little parafilm (this can be done at the end of the day). Record sampling information in field book and label bottle on bottles with sharpie (do not use regular felt tip pen). Place bottle in cooler (keep upright at all times) with either wet or dry ice. **USE A SIMPLE FIELD IDENTIFICATION CODE SYSTEM TO LABEL BOTTLE.** Also, use the letter F to note filtered samples (i.e., for nutrient samples place the term FNUTS on bottle).

- 3.2.1.7. In between stations in a day, rinse filtration equipment with a little dilute HCl and copious amounts of DI water. Place syringe, filter holder and collection cup into a “clean” field plastic bag for transport. Do not place them in the bag with unused equipment, use a “clean” bag. Take wet filter out of filter holder and either clean and reuse or place in “dirty” plastic bag. **KEEP CLEAN.**
- 3.2.1.8. At the beginning or end of day, clean equipment material with a little dilute HCL acid and copious amounts of DI water; put equipment into a new clean plastic bag.
- 3.2.1.9. Make sure all bottles are labeled and recorded in field book. Filtered nutrient and DOC samples need to be frozen immediately using dry ice (keep bottles upright at all times) or kept cold until end of day.

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Procedure No. P-13-66
Rev. 0 (09/03)

**FIELD SAMPLING PROCEDURES FOR
THE NEW JERSEY ALGAE INDICATORS PROJECT:
Use of Diatometer Artificial Substrates**

Prepared by: Karin Ponader, Diane Winter, and Donald Charles

**Field Sampling Procedures for the New Jersey Algae Indicators Project:
Use of Diatometer Artificial Substrates**

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 are used to assess relationships between extant water quality criteria (e.g., phosphorus and nitrogen concentrations) and overt signs of eutrophication. They are applied in a regulatory context as secondary criteria for identifying nutrient impairment. These indicators are based on an understanding of algal dynamics in New Jersey streams, and are able to distinguish between situations in which 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. This study was initiated in July 2000. Years 1 and 2 of this project were limited to development of algal indicators in the Piedmont physiographic province in New Jersey (Ponader & Charles, 2003). During the third year the study was expanded to include sites in the Highlands and the Ridge and Valley physiological provinces. Data from sites studied during all three years are currently being used to develop and test indicator metrics. The fourth year of the study extends the development of indicators to the Inner Coastal Plain physiographic province, using the same general approach and specific methods as used in the first three years (Charles et al., 2000). Nevertheless, because of the different geomorphology of the rivers in the NJ Coastal Plains (sand and clay river bottoms), significant changes and adjustments in the sampling design and the methods for collection of algal samples are necessary. The main changes are reflected in Algal Sampling procedures (Chapter 6.2) - using diatometers as artificial substrates.
- 1.3. The study sites correspond to 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 slides with no filamentous algae from diatometers deployed in portions of sampling sites with faster flowing current. These samples will analyzed for diatoms alone. If

applicable, 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 procedure is applicable mainly to NJ periphyton sampling sites of the Coastal Plain, 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.

2. REFERENCES:

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- 3.7. Ponader, K. and D. Charles. 2003. Understanding the Relationship Between Natural Conditions and Loadings on Eutrophication: Algal Indicators of Eutrophication for New Jersey Streams, Draft Final Report Year 2, The Academy of Natural Sciences, Philadelphia, PA.
- 3.8. Porter, S.D., T.F. Cuffney, M.E. Gurtz, and M.R. Meador. 1993. Methods for collecting algal samples as part of the national water-quality assessment program. Open-File Report 93-409. U.S. Geological Survey, Raleigh, North Carolina. 39 pp.
- 3.9. Velinsky, D. 2000. Syringe water sampling and filtration for the collection of filtered nutrient samples and unfiltered nutrient samples. PCER Procedure P-16-119. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 4 pp.
- 3.10. Velinsky, D., and J. DeAlteris. 2000. Benthic algae and sediment chlorophyll *a* preparation and analysis. PCER Procedure P-16-117. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 4 pp.

3. 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[™].
- 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 with ice for water chemistry samples and dry ice for biomass samples.
- 4.17. Strapping, clear and labeling tape.
- 4.18. ANS field sampling forms (attached).
- 4.19. Densimeter
- 4.20. Catherwood Diatometers.
- 4.21. Rope.
- 4.22. Slide Boxes.
- 4.23. 3- x 1-in microscope slides cleaned with 10% HCl and labeled.
- 4.24. Cinder blocks as anchors for diatometers.
- 4.25. Electrical tape for sealing biomass slide boxes.
- 4.26. Filter paper, pre-cut to fit in biomass slide boxes.
- 4.27. Zip-lock freezer bags for safe storing of biomass samples .
- 4.28. Latex gloves.

4. SAFETY PRECAUTIONS:

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

5. PROCEDURES:

- 6.1. Preparation of material for diatometer deployment and retrieval.

Use Catherwood Diatometers for samples of algal biomass (chlorophyll *a*, AFDM) and qualitative taxonomic analysis of diatom assemblages. For information on the general use of Catherwood diatometers please refer to Procedure No. P-13-01 “Use of the Catherwood Diatometer^(R) for the Study of periphyton communities in flowing and standing waters.” For diatometer deployment and retrieval please refer to section 6.3.

6.1.1. Slide preparation prior to fieldwork.

Please refer to Procedure No. P-13-01 “Use of the Catherwood Diatometer^(R) for the Study of Periphyton Communities in Flowing and Standing Waters” (6.2 Slide Preparation and Placement).

6.1.2. Deployment.

Prepare diatometers upon reaching the site, but prior to transfer to the river. Place the pre-cleaned slides in the slots of the diatometer. Wear lab/latex gloves while handling the cleaned slides to avoid transferring oils to the slide that may inhibit algal growth. Always place the slides in the diatometer in the same order. Prepare bottles for water chemistry samples and any natural substrate periphyton samples which will be collected. Sample bottles are wrapped with lab tape and labeled with the site name, date and type of sample.

6.1.3 Retrieval.

Prepare boxes for diatometer slides. Three boxes are required per site: two small, 5-slide boxes are used for the biomass slides and one large 25-slide box is used for the diatom assemblage slides. Label all boxes with the site name and the sampling date. The large box holds the diatom assemblage slides for both diatometers, and should be labeled as to which slides came from which diatometer. A convenient way to do this is to write “A” or “B” (or “1” or “2,” depending on the system used) in two places on the bottom of the slide box. The smaller biomass slide boxes need to have pre-cut pieces of paper (glass filter paper works well) put in one of the slide slots and wet with DI water to ensure the slides will not become too dry upon freezing. Prepare sample bottles for chemistry as for deployment.

6.2. Initial site characterization.

6.2.1. Location and assessment of site to determine sampling area.

6.2.1.1. 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. This is also the preferred location for algal sampling; however, the downstream side may be sampled if it is considered more representative of river habitat within the region.

- 6.2.1.2. Identify section of river to be sampled (“sampling reach” in NAWQA terminology) and division of sampling area into two sections.
 - 6.2.1.2.1. The length of the reach should be defined as 3 to 4 channel widths.
 - 6.2.1.2.2. The border of reach should be measured as the distance farthest up or downstream from bridge. The length of reach should be measured and recorded on the Site Description sheet.
- 6.2.2. Fill out heading and initial data on Site Description sheet.
 - 6.2.2.1. Record the site location, data, persons collecting, etc.
 - 6.2.2.2. Describe the site location and breakdown of reach into two sections.
 - 6.2.2.2.1. Indicate if site is upstream or downstream from bridge; if downstream, provide rationale for this choice.
 - 6.2.2.2.2. 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.
 - 6.2.2.2.3. If staff gage is present, record water level height.
 - 6.2.2.3. Record pH, conductivity, and water color using a pH/conductivity meter and a colorimeter.
 - 6.2.2.4. Continue making notes of other relevant information throughout sampling period.
- 6.2.3. Make measurements of river characteristics, to the extent it is practical (this may not be possible for sites with rapid flow or those which are very deep).
 - 6.2.3.1. Measure width and maximum depth (in meters).
 - 6.2.3.2. Estimate velocity of stream or river: fast, medium or slow.
 - 6.2.3.3. 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.
- 6.2.4. Take pictures of the habitat and/or the area sampled.
 - 6.2.4.1. Both upstream and down.
 - 6.2.4.2. Features relevant to local habitat quality (active erosion sites; local residential or commercial development).
 - 6.2.4.3. Typical substrate in streams with typical algal/macrophyte growth; whole areas of stream and riverbottom.
 - 6.2.4.4. Record pictures taken on Photograph form. If digital camera used, record image filename.

6.2. Algal sampling procedures.

Use Catherwood Diatometers for samples of algal biomass (chlorophyll *a*, AFDM) and qualitative taxonomic analysis of diatom assemblages. Two diatometers should be deployed per site, one per section. For information on the general use of Catherwood Diatometers and preparation of diatometer slides please refer to Procedure No. P-13-01 “Use of the Catherwood Diatometer^(R) for the Study of Periphyton Communities in Flowing and Standing Waters.” For preparation of material for diatometer deployment and retrieval please refer to section 6.1.

6.3.1. Deployment of diatometers

- 6.3.1.1. Per reach, find a location with open canopy cover, good flow and where the water is sufficiently deep. Avoid heavily shaded areas; select less shaded portions, if possible, so that diatometers receive direct sunlight for at least a part of the day. If many sites are rather shaded, then choose semi-shaded areas at all sites! Make sure to choose a section that is deep enough to provide sufficient water depth in case the water levels drop due to low flow conditions. It is very important that all physical conditions are consistent among sites so that results are comparable.
- 6.3.1.2. Estimate the length of rope needed. Make sure the rope is long enough (e.g., at least 2-3 times the water depth), but not too long either. Ideally the rope length should be long enough to allow water levels to rise, but at the same time short enough that the diatometer does not reach the shore if directed that way by the current. In case of a high flow event, the diatometer should not be held under water, because the rope is too short. It helps to observe the high flow marks on the riverbanks (mud on trunks of trees, leaves or other material deposited in over hanging branches etc.) in order to estimate how high the water could potentially rise and how long the rope should be.
- 6.3.1.3. Attach the diatometer to the cinder block, tying the end of the rope through one hole of the cinder block. Attach the other end of the rope to the diatometer. Make sure to tie knots that will hold when wet; the common bowline and double square knot work well.
- 6.3.1.5. Place the diatometer and anchor (cinder block) in the water. Make sure the diatometer is floating well in the current and does not come in contact with any surface obstructions (e.g., branches etc.). If necessary, adjust rope length.
- 6.3.1.6. Note on the field data sheet and in the site drawing the number of the diatometer (A or B or 1 or 2, depending on the numbering system used) and the exact location where it was placed. It is important to record this information, to be able to locate the diatometer at pick-up.

6.3.2. Retrieval of diatometer slides.

Allow a minimum of 14 and a maximum of 21 days exposure period. Ideally the slides should have a uniform periphyton cover at removal. Do not allow over-development to avoid sloughing of algal growth.

- 6.3.2.1. Record notes on physical characteristics on the Site Description sheet. Write down any changes in river conditions compared to the date when the diatometers were deployed (e.g., changes of water levels etc.). Take pictures and record image filenames. Record pH, conductivity, and water color.
 - 6.3.2.2. Carefully remove diatometers by cutting the rope and slowly lifting the diatometer out of the water. Minimize disturbance while the diatometer is still in water.
 - 6.3.2.3. Carry each diatometer to the riverbank and place it upright on a stable surface. Take a picture of each diatometer with slides before removing the slides.
 - 6.3.2.4. Remove slides by gently pushing each slide with one hand from the bottom and by gently grasping the edges of the slide.
 - 6.3.2.5. Start removing the biomass slides first. Use slides one and six (the two outer slides) for biomass samples. Gently slide the two biomass slides per diatometer into one slide box. Make sure to close the lid firmly and seal it with electrical tape. Use a separate biomass slide box for each diatometer. If the box is transparent, immediately wrap the box in aluminum foil, to protect it against sunlight. Put slide boxes in labeled Ziploc plastic bags and place them on dry ice. As soon as the biomass slides arrive in the laboratory they must be placed in the freezer.
 - 6.3.2.6. Finally, remove the four remaining diatom slides. Gently slide the four slides into the slide box. For the diatom assemblage slides the same slidebox may be used for the two diatometers from each site, but it must be indicated clearly on the bottom of the box which slides came from which diatometer (see also section 6.1). Close the lid and transport the box in an upright position. As soon as slide box arrives in the laboratory (ideally the same day), it should be placed in a clean place with the lid removed to allow the slides to dry completely.
- 6.3.3. If the substrate is of appropriate size (e.g., gravel of at least 1 cm diameter), collect qualitative samples for taxonomic analysis of diatom assemblages, ideally from both sections, or only one section if the substrate does not allow sampling in both sections. Also, if one section is too shallow, then only one diatometer may be deployed in one section and natural substrate may be used to collect algal samples in the other section.
- 6.3.3.1. Collect five or more rocks from each of the two sampling sections.
 - 6.3.3.2. 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, within the main water flow.

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.

6.3.3.3. Use brushes and a squirt bottle to remove algal growth from about 4 to 6 cm² 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 remove 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.

6.3.3.4. Pour the composite sample from each sampling reach section into a separate bottle; there will be two 125-ml bottles from each sampling reach that has sufficient rock substrate. Add buffered formalin as preservative; the final concentration should constitute 3 to 5% of the total sample (Moulton et al., 2000); closer to 3% if the sample is mostly diatoms. Label the bottle with sampling reach and section, date, collectors' initials, and number of rocks.

6.4. Substrate characterization.

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

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

6.4.1.2. 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.

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

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

6.4.2. If applicable, estimate the amount of bottom covered by various algal types using the EPA Periphyton Rapid Bioassessment method (Barbour et al., 1999). (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.)

6.4.2.1. 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.

6.4.2.1.1. 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.

6.4.2.1.2. 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.

6.4.2.1.3. 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.

6.4.2.2. Modifications to viewing bucket method.

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

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

6.4.2.2.3. 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 Procedure No. P-16-119, ANS Procedure for syringe water sampling and filtration for the collection of filtered nutrient samples and unfiltered nutrient samples.)

6.5.1. Collect water samples twice at each site: once at deployment of the diatometers and a second time at retrieval.

6.5.1. Collect water samples initially upon arrival at each site, before other collection activities are performed.

6.5.2. Prepare 2 field blanks for every 10 sites. Prepare these in a manner identical to a real sample, but instead of using water from a river or stream, substitute distilled water. There will be one filtered and one unfiltered field blank.

6.5.3. Every 10 sites, take duplicate samples, for quality control purposes.

6.5.4. Water chemistry sampling method.

6.5.4.1. Place paper towels on top of the work area.

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

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

- 6.5.4.3.1. Rinse the inside of the syringe, the syringe plunger and filter holder quickly with dilute acid and then thoroughly with distilled water.
- 6.5.4.3.2. Place the filter into the holder with forceps or tweezers.
- 6.5.4.4. Fill the syringe with clean stream water (collect sample water from below the surface of the water) or distilled water.
- 6.5.4.5. 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.
- 6.5.4.6. 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).
- 6.5.4.7. Make sure both bottles are labeled correctly and place directly in the cooler with wet ice.
- 6.5.5. Change the filter and clean the filter and the syringe 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.

- 6.6.1. Make sure all bottles are tightly sealed and properly labeled.
- 6.6.2. Place water chemistry bottles in Ziploc plastic 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.
- 6.6.3. Place algal sample bottles in the cooler.
- 6.6.4. Double bag ice in Ziploc bags to prevent leakage, and place around and on top of bottles to be shipped.
- 6.6.5. 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.
- 6.6.6. Close and seal cooler with strapping tape. Affix FedEx or other label to the lid of the cooler.
- 6.6.7. Ship samples for overnight delivery to arrive at the laboratory the next morning. If samples are not shipped, bring directly to the laboratory 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. ATTACHMENTS:

- 8.1. Examples of Sample Data sheets used; Site Description, Site Drawing, Section Description, Photographic record, Inorganic Substrate Components, Rapid Periphyton Survey Field Sheets (2 types).
- 8.2. Use of the Catherwood Diatometer ^(R) for the Study of periphyton communities in flowing and standing waters. PCER Procedure No. P-13-01, Rev. 1.

Attachment 8.1
SAMPLE DATA SHEETS

Diatrometer Deployment

Site Description

Site Name: _____ Site #: AN- _____

Date: _____ Time arrived: _____ Time

finished: _____

Collectors: _____

Weather/Temperature: _____

Velocity of stream (m/sec): _____; slow moderate fast

Average Width (m): _____ Temp (°C): _____ pH: _____

Reach Length (m): _____ Conductivity (ΦS): _____ water color _____

Site

Description: _____

Miscellaneous Notes:

Procedure No. P-13-66

Site Name: _____ Site #: AN- _____ Date: _____

Site Drawing:

Site Name: _____ Site #: AN- _____ Date: _____

Section 1:

Length of Section (m): _____ Velocity (m/s): _____; slow moderate fast

Densimeter:

of openings counted: _____

of openings *1.04: _____

Section 2:

Length of Section (m): _____ Velocity (m/s): _____; slow
moderate fast

Densimeter:

of openings counted: _____

of openings *1.04: _____

Photographs:

Site Name: _____ Site #: AN- _____ Date: _____

Picture #

Diatometer Pick-up

Site Name: _____

Site #: AN- _____ Diatometer site #: _____

Date: _____ Time arrived: _____ Time

finished: _____

Collectors: _____

Weather/Temperature: _____

Velocity of stream: slow moderate fast Average Width (m) _____

Temp (°C): _____ pH: _____ Conductivity (μS): _____ watercolor: _____

Site Description

Algae Biomass/Chlorophyll a (quantitative): "S" = section

of locations sampled: S1 _____ S2 _____ type of sediment sampled: _____

Method used: Turkey baster _____ Inverted petri-dish: _____

Diatoms(qualitative):

of locations sampled: S1 _____ S2 _____ type of sediment sampled: _____

Method used: Turkey baster _____ Inverted petri-dish: _____

Picture:

Procedure No. P-13-66

Attachment 8.2
ANS Procedure No. P-13-01

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-01
Rev. 1 (9/03)

**USE OF THE CATHERWOOD DIATOMETER[®] FOR THE STUDY
OF PERIPHYTON COMMUNITIES IN FLOWING AND STANDING
WATERS**

Prepared By: Frank Acker/Robin Davis

Use of the Catherwood Diatometer® for the Study of Periphyton Communities in Flowing and Standing Waters

1. PURPOSE:

This procedure describes the use of the Catherwood Diatometer® artificial substrate device as a means of sampling periphyton communities of flowing and standing waters.

2. SCOPE:

- 2.1. This procedure covers all aspects of deployment and retrieval of Catherwood Diatometers® (referred to below as diatometers) for the purpose of obtaining periphyton community samples from flowing or standing waters.
- 2.2. Periphyton samples obtained from diatometers can be used for algal community analyses (especially diatom community analyses), dry and ash-free weight determinations, pigments analyses, radioscope primary production measurements, and any other algal analysis techniques requiring either live, dried or preserved samples of periphyton. Samples are also usable for bioaccumulation studies of both organic and inorganic substances.
- 2.3. This procedure applies to personnel involved with the placement or retrieval of diatometers.

3. DEFINITIONS:

- 3.1. Periphyton refers to the communities of microorganisms found growing on natural and artificial substrates of streams, rivers, lakes and estuaries.
- 3.2. The Catherwood Diatometer® is a trademark registration of the Academy of Natural Sciences of Philadelphia filed June 25, 1953 with the United States Patent Office (Reg. No. 614,368 - current renewal effective to October 18, 1995).

4. REFERENCES:

- 4.1. Patrick, Hohn and Wallace. 1954. A new method for determining the pattern of the diatom flora.
- 4.2. APHA, AWWA, WPCF. 1985. Standard Methods for the Examination of Water and Wastewater. 16th ed.

5. APPARATUS/EQUIPMENT:

- 5.1. Diatometers employed by the Division of Environmental Research are routinely one of two sizes. The “standard” diatometer accommodates up to six 25 x 75 mm (1" x 3") glass microscope slides per instrument. A larger diatometer frame has been developed which accommodates up to six 76 x 102 mm (3" x 4") glass slides. Depending on the project requirements, sufficient diatometers of the proper size should be available for all station placements as well as an additional 10% as spares. Diatometers can be newly constructed or reconditioned as called for in the study protocol.
- 5.2. Glass slides as described above sufficient to fill all slots of the diatometers to be used. An additional 10% should be carried as spares.
- 5.3. 10% HCl solution for cleaning slides prior to use.
- 5.4. Diamond scribe.
- 5.5. Slide boxes or other receptacles to carry unexposed slides to the project site(s) and/or exposed slides taken from the diatometers.
- 5.6. Sufficient rope or cable for instrument attachment to anchors or stationary points of attachment (trees, bridges, etc.).
- 5.7. Anchors of sufficient weight to hold the instruments in the highest flow velocities expected at the study site. In lieu of anchors, stationary points of attachment such as trees, bridges, or pilings may be used.
- 5.8. Field sheets, notebook, pencil or pen (waterproof ink only).
- 5.9. Chain-of-custody forms.
- 5.10. Water quality instrumentation if called for (job specific).

6. INSTRUCTIONS:

- 6.1. Placement of Diatometers.
 - 6.1.1. At a given station or site, individual diatometers should be located in areas as free of surface obstructions as possible, positioned so that they will receive direct sunlight for at least part of the day. The instrument should be located so that it will be free-floating at all times, moored in deep enough water so that the frame will not touch bottom at any time. If replicate diatometers are to be used at a single station or site, great care should be taken to locate moorings so that each instrument is

subjected to as similar physical conditions as possible. Special attention should be given to water velocity. If a current meter is not available, areas of like current can be estimated by timing the movement of a floating object past fixed points of known distance apart. This method will result in an estimate of water velocity (e.g., meters traveled per second) which should be sufficient to match velocities among diatometer locations. If the intent of the use of multiple instruments at a single location or site is to sample a variety of current regimes, each instrument should be assigned a unique sub-station number and the respective velocities should be noted.

- 6.1.2. The locations of diatometers at other stations or sites should match as closely as possible those physical conditions prevailing at the other sites.
- 6.1.3. At the time of installation, all pertinent descriptions of the individual sites should be written into the project notebook and should include detail on the following primary considerations: light availability; water velocity; depth of water; distance from shore; upstream distance to objects which could provide “seed communities” of organisms.

6.2. Slide Preparation and Placement.

Slides to be used as the artificial substrates for periphyton attachment are labeled by means of a diamond scribe. Labels should be used on only one side of each slide, should be written across the narrow face dimension of the slide, and should occupy no more than one-quarter of the length of the slide. The label on each slide should include, as a minimum, the date of installation of the slide and station or sub-station number of the diatometer in which the slide is to be placed. For certain applications, it is useful to designate the position of the slide within the diatometer by placing a letter (a through f) on the slides intended for the individual slide locations (slots) with slide ‘a’ being placed in the slot farthest to the left (holding the diatometer upright with the front of the diatometer facing away from the investigator), slide ‘f’ in the slot farthest to the right. The label side of the slide should be placed to the right with the frame oriented as above. All slide labeling for a given site should be done at one time. Subsequent to labeling, all slides should be rinsed off with 10% HCl to remove any fingerprints or residues left from the manufacture of the slides. Once washed in acid, slides should only be handled on their edges. When the slides are properly loaded in the diatometer, the slide retaining clip is then placed in position and the instrument is placed gently in the water.

6.3. Exposure Period.

Ideally, the period of exposure of the slide substrates should be sufficient to allow the development of a uniform periphyton layer, yet not so long as to

allow over-development to the point where material is lost through detachment. A period of fourteen days has proved to be adequate in a wide variety of applications throughout the country, and can be used on new projects if compatible with the goals of the study element, and if sufficient material is developed without loss through detachment. Although the length of the exposure period may change from project to project, or within a project across seasons, it is imperative that the exposure periods of samples to be compared with one another be the same.

6.4. Retrieval of Slides.

6.4.1. At the time of removal of slides, the instrument should be lifted gently from the water and placed upright on a stable surface. The retaining clip is moved off the slide edges. Each slide may then be removed by carefully grasping the edges of the slide, moving it up and out of the retaining slots. If a slide is jammed in the slots, a pencil or other object may be used to push the slide out from beneath. In any case, the two flat surfaces of the slide should not be touched as this will invariably result in the loss of material from the slide. Slides can then be transferred to containers appropriate to the use the slides and attached material are to be put. As the etched slide label can no longer be read through the attached periphyton growth, it is necessary to carefully label a packing sheet at the time of slide removal, indicating the station and date represented by each slide (as well as the sub-station or slide position if called for by the study protocol). These packing sheets should accompany the slides to the Academy laboratories for use at the time of sample processing. Chain-of-Custody forms can be used for this purpose as long as the individual slides can be identified through the use of the form.

6.4.2. Note any unusual conditions seen on the slide in the field notebook or on individual field sheets. Note any changes in the physical aspects of each site from the time of deployment in the field notebook.

6.5. Successive Exposure Periods.

Depending on the study protocol, diatometers at specific locations may be used for consecutive exposure periods or may be replaced with new instruments. If the same diatometers are to be used for successive exposure periods, it is imperative to clean the instruments as thoroughly as practical before reloading with new slides. This can be done using a long-handled scrub brush of a design capable of reaching the inside corners of the slide box. The instrument should be brushed and rinsed until nearly all attached material has been removed. Once cleaned, the attachment ring and rope (or cable) should be inspected for soundness. The diatometer can then be loaded with a new set of labeled slides and gently placed in the water.

6.6. Tandem Rigging.

In certain applications it is useful to tie diatometers to one another in series. If this is done, a minimum distance of one meter should be used between instruments. Diatometers deployed for the purpose of metal or organic bioaccumulation studies should not be positioned behind other instruments as contamination from the metal or plastic parts of upstream instruments could affect results.

7. QUALITY CONTROL:

- 7.1. Use locations within study sites that conform to the criteria in section 6.1.
- 7.2. Choose locations at different sites that have the same physical characteristics of all other sites paying particular attention to light availability, water velocity, depth of water, distance from shore, and upstream distance to objects which could provide “seed” communities of organisms. Record observations and/or measurements for physical characteristics of each diatometer site in the field notebook.
- 7.3. Be certain that all instruments are either new or cleaned (section 6.5) before loading with slides.
- 7.4. Clean slides with 10% HCl to remove fingerprints or other residues prior to deployment.
- 7.5. At the time of retrieval of slides, note any changes in the physical characteristic of the site from those that were noted at the time of deployment. If there is indication that the diatometer has been physically disturbed or has not been submerged throughout the exposure period, this should be indicated in the field notebook as well as on the Chain-of-Custody forms for the affected set of slides.
- 7.6. If individual slides are broken or otherwise show evidence of unusual disturbance during the course of the exposure, this information should be written on individual field sheets or the field notebook.
- 7.7. Chain-of-Custody forms should accompany the exposed slides from the time they are removed from the diatometers until they are processed in the Academy laboratories.

8. REPORTING:

- 8.1. The notations in the field notebook, on individual field sheets and on the Chain-of-Custody forms constitute the records necessary for review during the analysis and report phases of the project. All entries in field notebooks, individual field sheets or Chain-of-Custody forms should be initialed or signed indicating the person(s) responsible for the notations.

9. RECORDS:

- 9.1. Field notebook(s).
- 9.2. Individual field sheets (if appropriate to the project).
- 9.3. Chain-of-Custody forms.

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-67
Rev. 0 (09/04)

**FIELD SAMPLING PROCEDURES FOR
THE NEW JERSEY ALGAE INDICATORS PROJECT:
Sampling method for collection of qualitative diatom samples and algal
biomass samples from epipsammic/epipellic habitat in the field**

Prepared by: Karin Ponader and Diane Winter

**Field Sampling Procedures for the New Jersey Algae Indicators Project:
Sampling method for collection of qualitative diatom samples and algal biomass samples
from epipsammic/epipellic habitat in the field**

1. PURPOSE:

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 are used to assess relationships between extant water quality criteria (e.g., phosphorus and nitrogen concentrations) and overt signs of eutrophication. They are applied in a regulatory context as secondary criteria for identifying nutrient impairment. These indicators are based on an understanding of algal dynamics in New Jersey streams, and are able to distinguish between situations in which nutrient concentrations are high due to natural environmental conditions and those that result from anthropogenic influences. Protocols are needed which 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.
2. This study was initiated in July 2000. Years 1 and 2 of this project were limited to development of algal indicators in the Piedmont physiographic province in New Jersey. During the third year the study was expanded to include sites in the Highlands and the Ridge and Valley physiological provinces. Data from sites studied during all three years were used to develop and test indicator metrics (Ponader & Charles, 2003). The fourth and fifth years of the study extended the development of indicators to the Coastal Plain physiographic province, using the same general approach and specific methods as used in the first three years (Charles et al., 2000). Nevertheless, because of the different geomorphology of the rivers in the NJ Coastal Plains (sand and clay river bottoms), significant changes and adjustments in the sampling design and the methods for collection of algal samples were necessary, using diatometers as artificial substrates (Ponader et al., 2003). During the current study year (fifth year), the Outer Coastal Plain physiographic province will be sampled with diatometers using the same protocols as last year. In addition, we are going to sample epipsammic (sand) and epipellic (silt) substrata, in order to explore differences in diatom assemblages and algal biomass collected from different substrates (e.g., artificial substrates (diatometers) versus natural substrates (sand/silt)). Therefore, development of this new sampling method and protocol for collection of sand and silt substrate was necessary as outlined in the following.
3. This protocol was developed specifically for this project, so the study sites correspond to NJ DEP sites monitored for water quality, benthic invertebrates and/or fish. Two types of algal samples are collected. The Algal Biomass Samples (ABS) are quantitative and are analyzed for chlorophyll *a*, and ash free dry mass (AFDM). The Diatom Composite Samples (DCS) are qualitative, representing diatoms from epipsammic (sand) and/or epipellic (silt) sediments taken from representative portions of the sampling sites. These samples are analyzed for diatoms alone.

2. SCOPE:

- 2.1. While this procedure is applicable mainly to NJ periphyton sampling sites of the Coastal Plain, 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. F. Acker, B. Russell and E. Hagan. 2002. Diatom cleaning by nitric acid digestion with a microwave apparatus. PCER Procedure P-13-42. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 13 pp.
- 3.2. Charles, D., D. Winter and M. Hoffman. 2000. Field sampling procedures for the New Jersey Algae Indicators project. PCER Procedure P-13-64. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 18 pp.
- 3.3. Kiry, P., D. Velinsky and A.-M. Compton. 1999. Determination of dry weight and percent organic matter for sediments, tissues and benthic algae. PCER Procedure P-16-113. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 3 pp.
- 3.4. Moulton, S.R., J.G. Kennen, R.M. Goldstein and J.A. Hambrook. 2002. Revised Protocols for Sampling Algal, Invertebrate and Fish Communities as Part of the National Water Quality Assessment Program. Open-File Report 02-150. U.S. Geological Survey, Reston, VA. 75 pp.
- 3.5. Ponader, K. and D. Charles. 2003. Understanding the Relationship between natural conditions and loadings on eutrophication: Algal indicators of eutrophication for New Jersey streams, Draft Final Report Year 2, The Academy of Natural Sciences, Philadelphia, PA.
- 3.6. Ponader, K., D. Winter and D. Charles. 2003. Field sampling procedures for the New Jersey Algae indicators project: Use of diatometer artificial substrates. PCER Procedure P-13-66. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 32 pp.
- 3.7. Velinsky, D. and J. DeAlteris. 2000. Benthic algae and sediment chlorophyll *a* preparation and analysis. PCER Procedure P-16-117. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA. 4 pp.

4. APPARATUS/EQUIPMENT:

- 4.1. Large plastic pan.
- 4.2. Petri-dish (ca. 47 mm diameter).
- 4.3. Spatula.
- 4.4. Nalgene bottles; 125 ml, and 250 ml.
- 4.5. Squirrt bottle.
- 4.6. Turkey baster.
- 4.7. Cookie cutter (preferably round shape).
- 4.8. Coolers with ice for diatom and biomass samples.
- 4.9. Strapping, clear and labeling tape.
- 4.10. Zip-lock freezer bags for safe storing of biomass samples.
- 4.11. Sharpies or other permanent markers.
- 4.12. Small plastic funnel.

5. PROCEDURES:

Preparation of material for sediment sampling.

Prepare bottles for samples which will be collected. Use 125-ml sample bottles for diatom samples, and 250-ml sample bottles for biomass samples (weigh bottles and record weight on bottles in the lab prior to sampling) . Bottles are wrapped with lab tape and labeled with the site name, date and type of sample.

At the site, fill one squirt bottle with river water for rinsing of the biomass samples which will be collected.

5.2. Algal sampling procedures.

If two diatometers are deployed per site, (e.g., one diatometer per section), composite ABS and DCS should be taken at each section from sand/silt habitat in addition to the diatometers. For best comparison, the sand/silt (S/S) diatom and biomass samples should be taken at the time of retrieval of the diatometers. Samples should be taken in the following order, to avoid disturbance of sediments:

- a) water chemistry samples
- b) S/S ABS and DCS samples

c) removal of diatometers.

5.2.1. Collection of epipsammic/epipellic diatom composite samples (DCS).

5.2.1.1. Per reach, find 3-4 sand/silt locations that are representative of the site, with respect to the following criteria: substrate, canopy cover, flow and water depth. Select portions of the reach that are comparable to the conditions where the diatometer is deployed.

5.2.1.2. Use a turkey baster (well rinsed with river water) to suck up the flocculent surface sediments (silt). At each location in a reach, fill the turkey baster once.

5.2.1.3. At each location in a reach, empty the turkey baster into the same 125-ml bottle. The end result will be one 125-ml bottle with a composite diatom sample from 3-4 locations across the reach.

5.2.1.4. Place the composite diatom sample bottle in the cooler on ice.

5.2.2. Collection of epipsammic/epipellic biomass samples.

Two different methods are suggested for collecting biomass samples from soft surface sediments; 1) the turkey baster method, and 2) the inverted petri-dish method. When arriving at the site, the field collectors should decide which method is more appropriate, depending on the prevailing substrate conditions at each site.

If the surface sediments are mostly composed of 1-10 mm of silt, accumulated over top of a sandy river bed, the turkey baster method should be used. If in contrast, the surface sediments are predominantly sand, with no visible accumulation of silt, the inverted petri-dish method should be used. If in doubt, both methods should be tried and the amount of flocculent material extracted (after shaking the bottle) should help in deciding which method works better. The method that provides more flocculent material should be used. Note on the field data sheets which method was used and why.

5.2.2.1. Turkey baster method.

5.2.2.1.1. Per reach, find 4-5 sand/silt locations that are representative of the site, with respect to the following criteria: substrate, canopy cover, flow and water depth. Select portions of the reach that are comparable to the conditions where the diatometer is deployed.

5.2.2.1.2. At each location in a reach, carefully push a ring with a defined area (e.g., cookie cutter) into the sediments without disturbing the sediments too much. Use a turkey baster (well rinsed with river water) to suck up the flocculent surface sediments (silt) within the area defined by the ring.

5.2.2.1.3. At each location in a reach, empty the turkey baster into the same 250-ml bottle. The end result will be one 250-ml bottle with a composite biomass sample from 4-5 locations across the reach.

5.2.2.1.4. Place the composite biomass sample bottle on ice in the cooler.

5.2.2.2. Inverted petri-dish method.

This procedure was modified after Moulton et al. (2002) ; 4.3.4 Sampling method for epipsammic/epipellic habitats.

5.2.2.2.1. Per reach, find 4-5 sand/silt locations that are representative of the site, with respect to the following criteria: substrate, canopy cover, flow and water depth. Select portions of the reach that are comparable to the conditions where the diatometer is deployed.

5.2.2.2.2. At each location in a reach, hold the lid of a small plastic petri-dish (about 47 mm diameter) upside down in the water; rub the inside of the lid to remove air bubbles.

5.2.2.2.3. Slowly turn the inside of the lid towards the sediments without disturbing the sediment.

5.2.2.2.4. Carefully and slowly press the lid into the sediment.

5.2.2.2.5. Slide the lid onto the spatula (fig. 3d in Moulton et al., 2002) to enclose a discrete collection. Holding the petri-dish tight against the spatula, carefully wash extraneous sediment from the spatula, and then lift out of the water.

5.2.2.2.6. Invert the lid and remove the spatula.

5.2.2.2.7. Rinse the sediment from the lid with stream water into either a flat tray or directly into the 250-ml sampling bottle using a funnel.

5.2.2.2.8. Repeat this collection procedure at 4-5 sampling locations in the reach. Collect all 4-5 samples in the tray, or in the 250-ml sampling bottle using a funnel.

5.2.2.2.9. If using the tray instead of the funnel to collect all 4-5 samples, pour the composite sample from the tray into the 250-ml sample bottle. Make sure to collect all sediment from the tray, rinsing thoroughly using river water. The end result will be one 250-ml bottle with a composite biomass sample from 4-5 locations across the reach.

5.2.2.2.10. Place the composite biomass sample bottle on ice in the cooler.

6. PREPARING SAMPLES FOR OVERNIGHT SHIPMENT/SAME DAY DELIVERY:

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

6.2. Place algal sample bottles in the cooler.

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

6.4. 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, giving a detailed description of each sample and what should be done with each upon receipt in the lab at ANS.

- 6.5. Close and seal cooler with strapping tape. Affix FedEx or other label to the lid of the cooler.
- 6.6. Ship samples for overnight delivery to arrive at the laboratory the next morning. If samples are not shipped, bring them directly to the laboratory the day on which they are sampled.
- 6.7. For treatment of samples upon arrival in the lab, follow the following methods, in order to determine diatom species composition as well as chlorophyll *a* and ash-free dry-mass:
 - 6.7.1. For further treatment of the biomass samples follow PCER Procedures P-16-117 and P-16-113 (Velinsky & DeAlteris, 2000; Kiry et al., 1999.)
 - 6.7.2. Diatom samples should be preserved directly in the field (if shipped overnight) or immediately at arrival in the lab, with buffered formalin as preservative. The final concentration should constitute 3 to 5% of the total sample. The diatom samples should undergo final treatment in the lab using PCER Procedure No. P-13-42 (Acker et al., 2002).

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. All sampling devices (turkey basters, spatulas, pans, etc.) used to collect algae from soft sediments should be cleaned as thoroughly as possible between each use.
- 7.3. 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.
- 7.4. 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 QA Officer.
- 7.5. 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 QA Officer.

2. Laboratory Protocols

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-48
Rev. 1 (4/02)

**SUBSAMPLING PROCEDURES FOR USGS NAWQA PROGRAM
PERIPHYTON SAMPLES**

Prepared by: Frank Acker and Benjamin Russell and Erin Hagan

Subsampling Procedures for USGS NAWQA Program Periphyton Samples

Frank Acker, Benjamin Russell, and Erin Hagan

1. PURPOSE

- 1.1. The Phycology Section of the ANSP Patrick Center for Environmental Research (PCER) analyzes three types of periphyton samples collected by the USGS National Water-Quality Assessment (NAWQA) program: Richest Targeted Habitat (RTH), Depositional Targeted Habitat (DTH) and Qualitative Multiple Habitat (QMH). This purpose of this protocol is to describe procedures for subsampling each of these samples in preparation for analysis of both diatoms and soft algae. Measurements of original volumes and subsample volumes of RTH and DTH samples must be precise and accurate because they will be used to calculate concentrations of algae on sampled substrates. Because of the multiple growth habits of algae (e.g., filamentous, single cell), QMH periphyton must be subsampled carefully to ensure that all algal forms are represented in the subsamples to be analyzed. The procedure outlined here, facilitated by database-generated forms, allows for efficient and accurate subsampling of USGS NAWQA periphyton samples.

1. SCOPE

- 2.1. While this subsampling procedure is applicable mainly to NAWQA periphyton samples, it can be followed for all algal samples where precise volumetric or qualitative subsampling is involved.
- 2.2. This procedure applies to personnel responsible for preparing subsamples of algae samples prior to taxonomic analyses.
- 2.3. Procedures involving quantitative samples pertain to both the RTH and DTH samples.
- 2.4. There is a special procedure involving the subsampling of samples with large amounts of sand, silt or other heavy material that can interfere with algal analysis. In this procedure, the liquid portion is subsampled by volume and the heavier material that is difficult to suspend is separated by mass.

2. REFERENCES

- 3.1. United States Geological Survey, National Water-Quality Assessment Program. 1997. Procedures for Processing NAWQA Algal Samples. Draft Manuscript. February 1997.

- 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, NC.

3. DEFINITIONS

- 4.1. **Quantitative** samples refer to those collected using the RTH and DTH sampling protocols. There is one component, microalgae, for each sample. RTH samples are designated by the letters “ARE” embedded in the middle of the NAWQA sample code; DTH sample codes have “ADE” near the middle.
- 4.2. **Qualitative** samples refer to those collected using the QMH sampling protocol. There are two components, micro- and macroalgae, for each sample. NAWQA sample codes have “QMH” near the middle, and typically have an “A” at the end for the macroalgae component and a “B” at the end for the microalgae component.

4. APPARATUS/EQUIPMENT

- 5.1. Distilled (DW) or reverse osmosis (RO) water.
- 5.2. Dispenser bottle for DW or RO water.
- 5.3. Beakers (100-ml beakers [1/sample], 250 ml).
- 5.4. Beaker holding box (24 slots).
- 5.5. Graduated cylinders (10 ml, 25 ml, 100 ml, 250 ml, 500 ml and 1 L).
- 5.6. 20-ml vials (1/sample).
- 5.7. Diamond scriber.
- 5.8. Protective clothing (gloves, lab coat or apron, eye protection).
- 5.9. Positive-draw fume hood.
- 5.10. Screen cloth (210- μ m mesh).
- 5.11. Screening apparatus.
- 5.12. Large plastic disposable weighing boats.
- 5.13. Analytical balance, capacity to 500 g, 0.1 g accuracy.
- 5.14. Spatulas.
- 5.15. Plastic disposable pipettes
- 5.16. Desktop computer networked to Phycology Section databases.

6. SAFETY PRECAUTIONS

- 6.1. Because samples are preserved in formalin (2-10%), subsamples should be made in a positive-draw fume hood to reduce exposure. Wear gloves and eye protection.
- 6.2. The concentration of formalin in samples varies; therefore be cautious and anticipate that some samples may have much higher concentrations than others.

7. METHODS

- 7.1. Print a “NAWQA Sample Volume/Subsample Form” (Figure 1) for each set of samples. This form is generated using the report “rpt_Sample_Subsample_Volume” in the PHYCLGY database. The printed form will contain sample ID’s for the set of samples you selected when printing the form. Compare data on sample bottle labels with those on the form to ensure that all samples in the set are present. The type of sample (e.g., RTH, DTH, QMH) is embedded in the NAWQA sample code (Client Sample ID) (see Definitions 4.1 and 4.2); consult these to determine the type of subsampling for each. Also, to facilitate the start of the diatom subsample processing, print a “Diatom Slide Preparation Form” (Figure 2) for the set of samples. This form is generated using the report “Diatom Prep Form (NAWQA)” in the PHYCLGY database.
- 7.2. Prepare containers for both the soft algae subsample (PRx) and diatom subsample (DTx) before beginning the procedure; x = 1, 2, 3, etc. Designate the first set of subsamples as PR1 and DT1. If more than one subsample is taken, designate them PR2, PR3, etc. and DT2, DT3, etc. For each PRx sample, etch the sample and subsample IDs onto the 20-ml glass vials with a diamond scribe (e.g., GS029131 PR1), and mark them on the corresponding plastic caps with a permanent marker. Prepare clean, tall 100-ml beakers marked with numeric IDs for DTx subsamples.
- 7.3. Pour the entire contents of each quantitative sample (RTH and DTH) into a graduated cylinder of appropriate size and measure the total volume of the sample. Record the volume in the “ANSP Sample Volume” column of the “NAWQA SampleVolume/Subsample Form.” Return the sample to the original container, avoiding any loss of sample (it may be necessary to “wash” the graduated cylinder with the liquid portion of the sample). It is not necessary to measure the volume of qualitative (QMH) samples.
- 7.4. Prepare soft algae (PRx) and diatom (DTx) subsamples:
 - 7.4.1. **All subsampling procedures should be performed in a positive-draw fume hood to avoid exposure to formalin.**
 - 7.4.2. For quantitative samples (RTH and DTH) with large amounts of sand and silt that will not remain in suspension, accurate volumetric subsampling is precluded and special procedures must be followed. Skip to section 7.5 for these procedures.
 - 7.4.3. For each quantitative sample (RTH and DTH), determine an appropriate amount of subsample. In general, 20 ml is subsampled for soft algae and 20-100 ml is

subsampled for diatoms. The more visible the algae and organic material, the less subsample is needed. If the total amount of the original sample is less than 50 ml, use about one-third for soft algae and one-half for diatoms. If there are visible growth forms (e.g., colonial spheres or filaments), macerate the soft algae sample (micro-blender, tissue grinder, etc.).

- 7.4.4. For each quantitative periphyton sample, suspend the algal material by shaking or swirling, and carefully pour the amount of PRx subsample determined in section 7.4.3 into a graduated cylinder. Transfer the subsample to its corresponding etched 20-ml bottle. Repeat the measurement procedure for the DTx subsample, and transfer the material to a prepared 100-ml beaker. Record the volume of each subsample on the “NAWQA Sample Volume/Subsample Form” (Figure 1). In addition, record the beaker ID for the diatom subsample on the “Diatom Slide Preparation Form” (Figure 2). Proceed to section 7.6.
- 7.4.5. For each component of the qualitative samples, create a subsample for soft algae using procedures in sections 7.4.3 and 7.4.4, however, without the maceration step. If there are visible macro forms, selectively add a portion of these to the subsample(s). There will be a diatom subsample for only one component; use the microalgae component if both components are available. Subsample the diatom subsample as in sections 7.4.3 and 7.4.4. Skip to section 7.6.

7.5. Subsample quantitative samples with heavy sediment.

- 7.5.1. Some samples contain substantial amounts of heavy particles that cannot be kept in suspension long enough for accurate quantitative volumetric subsampling. In such cases, the sand-size ($>210 \mu\text{m}$) and larger particles of sediment must be separated from the liquid fraction of the sample, and sediment and liquid fractions are subsampled individually. After both liquid fraction and sand fraction subsamples are taken, they are combined into a final subsample. Record all calculations on the “NAWQA DTH Subsampling Data Sheet” (Figure 3).
- 7.5.2. **Preparation of screening apparatus:** Place a clean weighing boat on the analytical balance. Set up the screening device with a clean piece of screen and set it on the weighing boat. Record the mass of the apparatus plus boat on the data sheet (Figure 3), and re-zero the balance. Remove screening apparatus and place on top of a large glass beaker.
- 7.5.3. **Suspend sand and sediment by vigorously agitating the sample:** Immediately pour this material through the screening device into the beaker. If the screening device becomes clogged, use a plastic spatula to keep the sediments suspended until all the liquid passes through the screen. Use liquid from the beaker to rinse sediment remaining in the sample bottle through the screen. The material left on the screen is the “sand fraction;” the liquid retained in the beaker is the “liquid fraction.”

- 7.5.4. **Determination of fraction amounts:** Place the screening apparatus with sand fraction onto the weighing boat on the balance. Record the mass of the sand fraction and remove the boat and screening bottle from the balance. Measure the volume of the liquid fraction in an appropriate graduated cylinder and record. Return the liquid fraction to the sample bottle.
- 7.5.5. **Determination of subsample proportions:** Shake the sample bottle containing the liquid fraction and use a plastic disposable pipette to measure out a liquid sample (see section 7.4.3) into a 10-ml graduated cylinder (typically, about 5 ml). Record the liquid fraction subsample volume. Place the graduated cylinder on the balance and re-zero. Calculate the proportion of the whole liquid fraction represented by the subsample volume (subsample volume divided by liquid fraction volume) and record. Calculate the mass of the sand fraction subsample to be taken (the above proportion multiplied by the total sand fraction mass) and record.
- 7.5.6. **Completing the subsample:** Using the plastic spatula, add the appropriate mass of sand fraction to the tared graduated cylinder on the balance. Once the calculated portion of sand is added, wash any sand from the edges by inverting the cylinder while capping it with your thumb. Measure and record the total subsample volume. Transfer the completed subsample to the appropriate container (a tall 100-ml beaker for diatom subsamples or an etched 20-ml vial for soft algae subsamples). Sand remaining can be rinsed into the subsample container with DW or RO. For soft algae subsamples, fill the vial with DO or RO water to 20 ml; calculate and record the dilution/concentration factor (DCF). For diatom subsamples, calculate and record the DCF, fill beaker with DW or RO water, set aside for digestion and record the beaker number on the “Diatom Slide Preparation Form” (Figure 2).
- 7.5.7. **Cleanup:** When finished with subsampling, carefully return the sand fraction to the sample bottle. Record the total volume of sample removed. Remove the screen from the screening apparatus and place in soapy water to soak. Clean all glassware and the screening apparatus with hot, soapy water and rinse with DW or RO water.
- 7.6. **Enter data.** Complete filling-out the “NAWQA Sample Volume/Subsample Form” (Figure 1) and add it to the subproject “Sample Tracking and Subsampling” folder (See ANSP Protocol P13-58). Enter subsample data in PHYCLGY database tables, as described below.
- 7.6.1. Enter data for the following fields into the “Sample Volumes/Areas” table. This table will already contain a record for each sample with the sample identification fields filled out. This information was added to the table during the log-in process (see Protocol P-13-47).
- 7.6.1.1. **ANS vol (ml)** -- the amount of sample (in ml) received at ANSP (column labeled “ANSP Sample Volume” on paper form).

7.6.1.2. **Date ANS Samp Vol Measured.**

7.6.1.3. **ANS Samp Vol Measured By** -- Worker ID number (from “Worker Name” table) of the person who measured the sample volume.

7.6.2. Table “Subsample Information”. Enter data for diatom and soft-algae subsamples, each as a separate records.

7.6.2.1. **SampleID** -- the ANSP sample code (e.g., GS029131).

7.6.2.2. **SubSampleID** -- distinguishes between diatom (DTx) and soft algae (PRx) subsamples.

7.6.2.3. **Dilution/Concentration Factor** – the number needed to multiply the subsample volume by to get 20 ml (record as 1 if no dilution or concentration or if qualitative subsample).

7.6.2.4. **WorkerID** – Worker ID number of the person who did the subsampling; located in the Worker Name table of the NADED database.

7.6.2.5. **Date Subsampled.**

5. **QUALITY ASSURANCE/QUALITY CONTROL**

- 8.1. These procedures were developed by the ANSP as suggested in the USGS NAWQA Program protocols and contract documents. The specific procedures (including type of glassware, amounts of subsamples, etc.) evolved over a 5-year period. The procedures for samples with large amounts of sand or heavy sediment were developed by the USGS, adapted by the ANSP with little modification and have been used since 1997.
- 8.2. Because algae are microscopic, the possibility of contamination of samples is great. Laboratory rooms where raw samples are subsampled should be kept as clean as possible. Lab bench surfaces should be kept clean and free of debris.
- 8.3. Quantitative samples need to be mixed well during subsampling (possibly blended), to avoid clumps caused by natural growth forms (colonies, filaments, etc.).
- 8.4. The appropriate size of graduated cylinder for measuring samples and subsamples is critical. The sample should be at least one-third the capacity of graduated cylinder and the units of the graduated cylinder should allow estimation to the nearest milliliter (finer for the small graduated cylinders used to measure a portion of a subsample).

NAWQA Sample Volume/Subsampling Form: ANSPGS9901SB

Subproject ID: ANSPGS9912PR

			ANSP	Date		Volume	Volume		Sub-
		Subsample	Sample	Measured	Measured	Subsampled	Subsampled	Date	sampled
Sample ID	Client Sample ID	ID	Volume (ml)	(ANSP)	By	Periphyton (ml)	Diatoms (ml)	Sampled	By
GS021783	OZRK0997ARE0001B								
GS021785	OZRK0997AQE0001A								
GS021787	OZRK0997AQE0001B								
GS021793	OZRK0997ARE0002B								
GS021795	OZRK0997AQE0001A								
GS021797	OZRK0997AQE0002B								

Data Entered By: _____ / /

Confirmed By: _____ / /

Figure 1. NAWQA Sample Volume/Subsampling Form.

Diatom Slide Preparation Form - NAWQA					
For use with Protocol P-13-42 and samples collected for the U.S.G.S National Water Quality Assessment Program					
Phycology Section - Patrick Center for Environmental Research - The Academy of Natural Sciences					
Project Name: USGS NAWQA Algae CO-OP Year 3		Project ID: GS708230		ANSP Account Number: 708-2302	
Subproject ID: ANSPGS0109PR		Subproject Name: New England Coastal Basins (NECB 2001) USGS NAWQA			
Digestion Method: Microwave-Nitric Acid (P-13-42)				Digested By: _____ Date: ____/____/____	
Decant Dates:		1	2	3	4
		5	6	7	
Slide Prep. Method: Strewn Mount		Settled By: _____			
Mounting Medium: Naphrax		Mounted By: _____			
Sample ID	Client Sample ID	Beaker #	Microwave Vessel #	ul dripped	Estimates
GSN87110	NECB0801ARE0001B	_____	_____	_____	_____
GSN87114	NECB0801ADE0001B	_____	_____	_____	_____
GSN87116	NECB0801AQE0001B	_____	_____	_____	_____
GSN87117	NECB0801AQE0001A	_____	_____	_____	_____
GSN87118	NECB0801AQE0001C	_____	_____	_____	_____
GSN87128	NECB0801ARE0002B	_____	_____	_____	_____
GSN87130	NECB0801ADE0002B	_____	_____	_____	_____
GSN87132	NECB0801AQE0002A	_____	_____	_____	_____
GSN87133	NECB0801AQE0002B	_____	_____	_____	_____
Remnants of Original Samples Transmitted By: _____ Date: ____/____/____ To (location): _____					

Figure 1. Diatom Slide Preparation Form - NAWQA.

ANSP-____-____-____-DS

NAWQA DTH Subsampling Data Sheet

SampleID:_____ SubsampleIDs:_____

Date:_____

____/____/____

NAWQA ID:_____

Init:_____

1. Mass of weighing boat + screening apparatus: _____g
2. Sand fraction mass: _____g
3. Liquid fraction volume: _____ml
4. Liquid fraction subsample volume: _____ml
5. Proportion of liquid fraction represented by subsample: _____
(#4 divided by #3)
6. Mass of sand fraction to be taken: _____g
(#5 multiplied by #2)
7. Total subsample volume: _____ml

Subsample ID: DT1

Beaker #:_____

8. Diatom subsample DCF:

New subsample volume (20 ml) / Total subs. volume (#7)= _____

9. New sand fraction mass: _____g
10. New liquid fraction volume: _____ml
11. Liquid fraction subsample volume: _____ml
12. Proportion of liquid fraction represented by subsample: _____
(#11 divided by #10)
13. Mass of sand fraction to be taken: _____g
(#12 multiplied by #9)

14. Total subsample volume: _____ml Subsample ID: PR1

15. Periphyton subsample DCF:

New subsample volume (20ml) / Total subs. volume (#13)= _____

Total volume removed from sample: _____ml

Figure 3. NAWQA DTH Subsampling Data Sheet.

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Procedure No. P-13-42
Rev. 1 (4/02)

**DIATOM CLEANING BY NITRIC ACID DIGESTION WITH A
MICROWAVE APPARATUS**

Prepared by: Frank Acker and Benjamin Russell and Erin Hagan

Diatom Cleaning by Nitric Acid Digestion with a Microwave Apparatus

Frank Acker, Benjamin Russell and Erin Hagan

1. PURPOSE

- 1.1. To identify and enumerate diatoms accurately at the species and variety levels, it is necessary to remove both extracellular and intracellular organic matter from the siliceous frustules of diatoms and other material in the sample. Removing the organic matter is necessary so that all details of diatom structures necessary for taxonomic identification are clearly visible. This protocol describes a method for removing organic material from a sample by digesting it with nitric acid in a microwave apparatus.
- 1.2. Traditional nitric acid digestion methods utilize a hotplate to heat samples and acid in open beakers. The procedure described herein takes advantage of a microwave apparatus to heat the acid / sample mixture in closed containers. This procedure produces a cleaner sample (more complete digestion) with less contamination, and is safer and more convenient (much less use of acid).

2. SCOPE

- 2.1. This procedure is applicable for cleaning diatoms from a wide variety of samples including, but not limited to, periphyton samples from diatometer slides or other artificial substrates, collections from natural substrates, surface sediment or sediment core samples, and net or whole water collections of phytoplankton. Material to be cleaned may be in a moist, dry or preserved state. If material to be processed contains preservatives or other chemical substances, refer to the cautionary notes in sections 5 and 7.14.
- 2.2. This procedure applies to personnel responsible for preparing diatom slides for taxonomic or community analysis purposes.
- 2.3. This procedure applies to the use of Advanced Composite Vessels (CEM Corporation).
- 2.4. The procedure for diatom sample digestion using nitric acid and a microwave apparatus has been employed by the ANSP since 1992. In 1998, Lined Digestion Vessels were replaced by Advanced Composite Vessels because of their durability. The procedures described above evolved from nitric acid/hotplate digestions performed at the ANSP for over 40 years. The acid/hotplate procedures are described in ANSP Protocol No. P-13-02 and may be used occasionally for single samples.
- 2.5. If samples are essentially free from organic detritus, and critical taxonomic work is not required (e.g., multiple phytoplankton samples where the taxonomy is well known), this nitric acid digestion method may not be necessary. A hydrogen peroxide method (not described here) or burn mount procedure may be considered

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in these cases. However, for the vast majority of applications, the nitric acid digestion method is recommended.

3. REFERENCES

- 3.1. Acker, F.W., D.M. Walter, N.A. Roberts and D.F. Charles. 1993. Microwave Digestion of Diatom Samples. Poster presentation given at the 12th North American Diatom Symposium, 23-25 September 1993, Delta Marsh, Manitoba, Canada.
- 3.2. American Public Health Association, American Water Works Association, Water Environment Federation (APHA, AWWA, WEF). 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed.
- 3.3. J.T. Baker Chemical Company. Saf-T-Training Manual and Tests.
- 3.4. CEM Corporation. Instructions for Use of Lined Digestion Vessels (P/N 323000 Rev. 0).
- 3.5. CEM Corporation. Instructions for Use of Advanced Composite Vessels (P/N600214 Rev. 1).
- 3.6. Patrick, R. and C. Reimer. 1967. Diatoms of the United States. Vol 1. Monograph No. 13, Academy of Natural Sciences of Philadelphia. 688 pp.
- 3.7. PCER, ANSP. 2002. Analysis of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples. Protocol No. P-13-39.
- 3.8. PCER, ANSP. 2002. Log-In Procedures for USGS NAWQA Program Algal Samples. Protocol No. P-13-47.
- 3.9. PCER, ANSP. 2002. Subsampling Procedures for USGS NAWQA Program Periphyton Samples. Protocol No. P-13-48.
- 3.10. PCER, ANSP. 1988. Diatom Cleaning by Nitric Acid Digestion. Protocol No. P-13-02.
- 3.11. PCER, ANSP. 1994. Subsampling and Determination of Wet and Dry Weights of Lake Sediment Samples. Protocol No. P-13-43.
- 3.12. PCER. 1990. Laboratory Safety Manual

4. DEFINITIONS

- 4.1. **Digestion** in this procedure refers to the solubilization of organic material by strong acid oxidation.
- 4.2. Diatom cells, called **frustules**, are composed of two **valves**. They have a siliceous structure, the features of which are used for taxonomic identification.

5. APPARATUS/EQUIPMENT

- 5.1. Positive-draw fume hood.
- 5.2. Safety glasses.
- 5.3. Acid-impervious hand protection.
- 5.4. Laboratory coat or apron, acid resistant.
- 5.5. Microwave apparatus (CEM Model MDS-2100):
 - 5.5.1. 0-950 watts (1% intervals).
 - 5.5.2. Controlled and monitored temperature (fiber optics) and pressure.
 - 5.5.3. Programmability for different cycles of temperature, pressure, and time at various temperature and pressure combinations.
 - 5.5.4. Rotating turntable.
- 5.6. Closed microwave digestion vessel [see Figure 1 “Standard Advanced Composite Vessel (Cross Section)”]:
 - 5.6.1. Vessel liner (Teflon PFA[®]).
 - 5.6.2. Vessel liner cover (Teflon PFA[®]).
 - 5.6.3. Vessel cap (reinforced polyetherimide, microwave “invisible”).
 - 5.6.4. Thread ring (reinforced polyetherimide, microwave “invisible”).
 - 5.6.5. Sleeve (advanced composite material, microwave “invisible”).
 - 5.6.6. Rupture membrane (Teflon PFA[®]).
 - 5.6.7. Vent tube (Teflon[®]).
 - 5.6.8. Vent fitting (Teflon PFA[®]).
 - 5.6.9. Ferrule nut (Teflon PFA[®]).
- 5.7. Digestion vessel for temperature and pressure control and monitoring; see Figure 2 “Advanced Composite Vessel for Pressure and Temperature Control.” Vessel liner, vessel cap, thread ring, sleeve, rupture membrane, vent fitting and ferrule nuts as in sections 5.6.1, 5.6.3, 5.6.4, 5.6.5, 5.6.6, 5.6.7 and 5.6.8, respectively.
 - 5.7.1. Vessel liner cover (Teflon PFA[®]) with exhaust, temperature and pressure ports.
 - 5.7.2. Thermowell (Pyrex with Teflon coating).
- 5.8. Reagent grade nitric acid (~70%).
- 5.9. Potassium dichromate (crystal form).
- 5.10. 100-ml tall glass beakers.
- 5.11. Reverse osmosis water (RO) or distilled water (DW).
- 5.12. Faucet siphon apparatus.

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- 5.13. 20-ml glass vials; caps with coned liners.
- 5.14. Vial labels.
- 5.15. Diamond scribe.
- 5.16. Wash bottle for RO or DW.
- 5.17. Single-edged razor blades.
- 5.18. pH indicator paper.

6. SAFETY PRECAUTIONS

- 6.1. Nitric acid is an extremely hazardous reagent. As a strong acid oxidizer it can cause severe burning of exposed skin and clothing. At room temperature, concentrated nitric acid produces intense fumes when exposed to open air.
- 6.2. Any concentrated nitric acid containers open to the air must be contained within a positive-draw fume hood at all times. There are no exceptions to this rule.
- 6.3. Personnel are required to wear safety glasses, protective gloves and lab coats at all times when handling concentrated nitric acid. This is especially important when handling/venting the digestion vessels.
- 6.4. When samples are delivered to the Diatom Preparation Laboratory, personnel who will work with the samples must be informed if any preservatives have been used in the samples (e.g., formaldehyde, Lugol's solution, glutaraldehyde, etc.). This information should be on the shipping forms included with the samples or affixed to the shipping container. Consult the Material Safety Data Sheets (MSDS) located on the 2nd floor of ANSP near the Diatom Preparation Laboratory for information on any of the above preservatives and how to handle them properly. Unexpected, violent and/or noxious reactions can occur during the cleaning procedure if nitric acid is mixed with other chemical substances. **Samples are to be rejected by laboratory personnel if their collection history and content are not fully known.**
- 6.5. **Never** use an Advanced Composite Vessel without a composite sleeve.
- 6.6. **Never** install more than one rupture membrane in the vessel cover.
- 6.7. Prior to use, all vessel components must be dry and free of particulate matter. Drops of liquid or particles will absorb microwave energy, causing localized heating which may char and damage vessel components, leading to possible vessel failure.

7. METHODS

Algal material that requires processing for diatom analysis comes to the preparation lab in several forms, depending on the collection protocol. Diatom collections may come from glass periphytometer slides, tile or other artificial substrates, phytoplankton suspensions, culture material, dried herbarium material and lake sediments. To produce a diatom slide, regardless of the kind of collection, the lab technician must remove all organic materials from the sample so that diatom frustules can be identified. This may

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require a preliminary examination of the raw sample with a microscope to determine the proper amount to digest. Proper cell densities for diatom slide analysis are described in Protocol No. P-13-39. Procedures for other sample types are discussed below, including samples with preservatives (sections 5 and 7.14), and samples with carbonates (section 7.14). This procedure should be started only after all samples are logged in (refer to Protocol No. P-13-47).

Record sample digestion data (Beaker #, Microwave Vessel #, notes) on the appropriate “Diatom Slide Preparation Form” (see, for example, Figure 2 in ANSP Protocol No. P-13-48). For most types of samples this form will have been generated during the process of preparing subsamples, and placed in the “Diatom Analysis” folder. Sample digestion data are not added to a computer database. If a “Diatom Slide Preparation Form” has not been generated, it can be printed using a report in the PHYCLGY database. There are different reports for different types of samples and projects (e.g., diatometer, survey, NAWQA, sediment), all of which contain “Diatom Prep Form” as the first part of their name. Confirm that all samples to be processed are recorded on the form before proceeding.

- 7.1. **Glass slides with attached periphyton (e.g., diatometer slides).** Based on the thickness of attached growth, choose one or more slides from the same sampling station or site for the cleaning process (set aside those not used in the cleaning process for eventual curation as “uncleaned material”). Place the chosen slides from individual sites in a single, numbered 100-ml beaker. Record the number of slides to be processed and the respective beaker number on the “Diatom Slide Preparation Form”. Fill each beaker with distilled water so as to completely immerse the slides, and allow the slides to soak for a minimum of 12 hours. After the soaking period, use a disposable single-edged razor blade to scrape algae from the slides (including the slide edges) into the beaker. Then dip the slides and razor blade in the water-filled beaker to transfer remaining scraped material from the slides. Using a wash bottle filled with distilled water, carefully rinse any material still adhering to the slides or the razor blade back into the beaker. At this point, cross check the etched label of the slide with the information on the “Diatom Slide Preparation Form” to ensure that the slides have been transferred from the proper field sites and that the date of installation is correct. If at this stage there are any discrepancies, they should be noted on the “Diatom Slide Preparation Form” and resolved. Record the number of sides scraped for each sample.

Allow the material in the beakers to settle for a period of at least 8 hours without being disturbed. Then siphon off the supernatant liquid without disturbing any of the material that has settled to the bottom. In the siphoning process, the tip of the siphon should be placed just beneath the water’s meniscus, and moved slowly down as the water level drops, to prevent loss of material through water column turbulence and contamination of the siphon tip. As much water as possible should be removed without disturbing the sedimented material. Proceed to section 7.5.

- 7.2. **Water suspended samples (e.g., phytoplankton samples, culture material).** If necessary, concentrate samples by siphoning water off undisturbed samples as described in section 7.1 until the sample contains approximately 20 ml of water and

associated material. If a quantitative analysis is required, the original volume of the sample must be recorded. If the sample is too large or too dense to clean in its entirety, a subsample should be taken and processed. Volumes of the subsample and the original sample must be recorded on a "Sample Volume/Subsample" form (e.g., See Protocol P-13-48). Then transfer the sample to 100-ml beakers as in section 7.1. Proceed to section 7.5.

7.3. **Sediments (e.g., bottom substrate samples or sediment core samples).** Transfer approximately 0.5 to 1.0 cc of either moist or dry sediment to a 100-ml beaker. If dry, a small amount of water may be added to the sample (approximately 10 ml) to hasten disaggregation. If the sample is to be analyzed quantitatively, record the wet weight, volume, or dry weight of the sample to be processed, according to the specifications of the study protocol. Unless otherwise specified, use procedures and forms in Protocol No. P-13-43. Proceed to section 7.5.

7.4. **Other.** Samples from other sources (e.g., natural rock substrates; soft sediments from rivers) may also be digested using this procedure. As in the above cases, it is important to begin with a concentrated sample to which nitric acid can be added safely in order to process a large enough sample to yield a sufficient concentration of cleaned diatoms for the required analyses. Care should always be taken to ensure that violent reactions will not take place upon the addition of acid. For quantitative analyses, the weight, area or volume of the original sample should be noted as required by the protocol.

7.5. **Addition of nitric acid. WARNING. THE FOLLOWING PROCEDURE IS TO BE PERFORMED ONLY IN A POSITIVE-DRAW FUME HOOD. TECHNICIANS ARE REQUIRED TO WEAR SAFETY GLASSES AND PROTECTIVE GLOVES!**

Assemble part of the digestion vessel before adding acid (sections 7.6.1 and 7.6.2). Place sample into the vessel liner portion of a microwave digestion vessel. Add concentrated nitric acid to the sample in the vessel liner; add an amount of acid equal to the amount of sample. If the sample contains a very high amount of organic material, more acid can be added (routinely the sample is in a 10-ml water matrix requiring the addition of 10-ml nitric acid). Initially, add acid very slowly and with great caution, anticipating that an unexpected reaction may take place. After determining that there is no possibility of a violent reaction, slowly and cautiously add the remainder of the acid to the samples.

7.6. **Preparation, assembly of digestion vessels and connecting to the microwave apparatus.**

7.6.1. Before the assembly of the digestion vessels, new rupture membranes must be seated in the vessel liner covers as illustrated in Figure 3, "Installation and removal of rupture membrane." Make sure that there is only one rupture membrane seated in each vessel liner cover.

7.6.2. Once the rupture membrane is seated, turn the vent stem back into the vessel liner cover until hand tight. Do not apply excess pressure or use a wrench to

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tighten the vent stem! Sections 7.6.1 and 7.6.2 should be completed prior to the acid addition (section 7.5).

- 7.6.3. Place the vessel liner cover (with rupture membrane and vent stem installed) on top of the vessel liner.
- 7.6.4. Thread the vessel cap onto the thread ring (by turning in a clockwise direction) until hand tight. As with the vent stem assembly, do not apply excess pressure or use a wrench to tighten the vessel cap!
- 7.6.5. Insert the vent tube into the vessel by threading through the ferrule nut into the vent fitting (located on top of the vessel cover extending above the vessel cap).
- 7.6.6. Place the complete vessel assembly into a turntable, orienting the vent tube towards and into the collection vessel at the center of the turntable. Record position of vessel (in turntable) on the Diatom Slide Preparation Form.
- 7.6.7. Complete the vessel assembly by placing an advanced composite sleeve over the liner and under the thread ring.
- 7.6.8. For the remaining vessels repeat steps 7.6.1 to 7.6.7. Note the differences for the vessel with the temperature and pressure controls (assembly is similar but with a different type of vessel liner cap and cover); see Figure 4, "Installation of rupture membrane in vent fitting of Advanced Composite Vessel with Temperature/Pressure control cover."
- 7.6.9. Place turntable with vessels into the microwave apparatus cavity on its drive lug. Turn on microwave apparatus and rotate the turntable. After confirming the operation of the turntable, rotate so that the vessel with the temperature and pressure sensors is at 12:00 (as looking into the microwave cavity).
- 7.6.10. Bleed and connect pressure sensing line:
 - 7.6.10.1. Using keypad controls and menu system, rotate turntable to 9:00.
 - 7.6.10.2. Turn pressure valve (outside left of microwave cavity) to **open**.
 - 7.6.10.3. Tap pressure sensing line to get air bubbles to connection end.
 - 7.6.10.4. With the syringe filler (outside left of microwave cavity), flush air bubbles out of the pressure sensing line.
 - 7.6.10.5. Connect pressure sensing line to the pressure port on the temperature and pressure vessel liner cover (use only hand pressure!) and place pressure sensing line in center post of the turntable; see Figure 5, "Routing of pressure sensing line and fiber optic temperature probe."
 - 7.6.10.6. Turn pressure valve to neutral.
- 7.6.11. **Connect fiber optic temperature probe:** Without bending, place fiber optic probe carefully (note: it is glass!) into the thermowell of the temperature and pressure vessel liner cover and connect (use only hand pressure for the connection!). Thread probe into center of the turntable, similar to the pressure

sensing tube; see Figure 5, "Routing of pressure sensing line and fiber optic temperature probe."

7.7. Configuring the microwave apparatus.

7.7.1. From the main menu, choose either to load a preexisting program (current program "Diatom New Vessel") or to enter new operating conditions (and new programs) from the keypad.

7.7.2. In the view mode, use the arrow keys and numeric keypad to set the following operating conditions:

Cycle 1 - 25% power, 20 PSI, 5 min @ pressure;

Cycle 2 - 80% power, 60 PSI, 5 min @ pressure;

Cycle 3 - 90% power, 100 PSI, 20 min @ pressure;

Adjust power settings for number of vessels by reducing 3% for each vessel less than a full tray (12 vessels);

Record additional information in the "Sample Information" screen.

7.7.3. Make sure printer is connected and on and print out operating conditions and sample information.

7.8. Start the microwave apparatus; the program will take about 60 min.

7.9. It is crucial that the operator monitor the temperature and pressure controls (readings printed every 30 sec) during the course of the digestion. In stages the temperature will rise from room temperature to about 170°C (up to 90°C in Cycle 1; up to 140°C in Cycle 2; up to 170°C in Cycle 3) and pressure will go from 0 psi to 100 psi (0-25 psi in Cycle 1, 25 to 70 psi in Cycle 2; 70-100 psi in Cycle 3). In the first two cycles, the rise will be even for 5-10 min and then hold near the maximum for 5 min; in Cycle 3 the rise will be fast (within a minute or two) and then hold for 20 min (@ 90-100 psi and 160-170°C). Deviations from this controlled rise in temperature and pressure could indicate a break in a vessel or problem sample reaction. The digestion should be stopped immediately if either the temperature or pressure does not rise evenly through the cycles.

7.10. Remove samples from the microwave apparatus (after temperature and pressure conditions are back to normal levels; ~30-45 min):

7.10.1. Place the vessel with the temperature and pressure controls at 9:00.

7.10.2. Vent the vessel with the temperature and pressure controls by turning the vent fitting counter- clockwise. Note: gloves, lab coat and eye protection should be worn during venting of vessels.

7.10.3. Carefully take out the fiber optic probe and place in one of the holes near the top of the microwave cavity.

7.10.4. Remove pressure sensing line and place away from the turntable.

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- 7.10.5. Remove turntable from microwave cavity and place in fume hood; carefully vent each of the vessels by slowly turning the vent fitting counter-clockwise.
- 7.11. Remove vessel caps, vessel liner covers and transfer samples to tall, 100-ml beakers, washing liner with DW or RO from wash bottle. Fill beaker with DW; be sure to check beaker numbers on “Diatom Slide Preparation Form.”
- 7.12. **Decanting procedure.** After 8 hours, siphon off the supernatant in a manner similar to that described in section 7.1. DW is again added to beakers. Repeat this settling and siphoning procedure at least five more times or until the pH is similar to that of the DW or RO (above 6.5). Note date of each decant on the “Diatom Slide Preparation Form.”
- 7.13. **Transfer procedure.** Carefully swirl the cleaned material remaining in the bottom of each beaker after the final siphoning and then pour it into a 20-ml glass vial which has been previously labeled with Sample ID and SubSample ID. (Labels should be made on the side of the vial using a diamond scribe and on the cap using an indelible marker). Using a wash bottle containing DW or RO water, wash any remaining material adhering to the beaker sides into the vial, and bring the volume of each vial to exactly 20 ml. Cap the vial and store with others until ready to make slides. Initial and date the “Diatom Slide Preparation Form,” put it in the “Diatom Analysis” folder, and keep the folder with the samples.
- 7.14. **Precautions.** Samples containing preservatives or other chemicals should be clearly identified at the time of submission to the diatom laboratory. If chemical additives are suspected, but not indicated on the preparation form, technicians should request confirmation from the Principal Investigator or the Project Leader as to the contents of the samples. If the samples are known to contain formaldehyde, Lugol’s solution, glutaraldehyde or any other noxious material, a dilution step should be performed by the addition and siphoning of DW or RO similar to that described in section 7.12 (excluding the consideration of pH) until it is safe to assume that the added chemical or chemicals have been significantly diluted. Samples containing significant amounts of carbonate will tend to bubble and sputter on the initial addition of acid. In this case, acid addition should be suspended immediately until all evidence of a reaction has ceased.
- 7.15. **Addition of potassium dichromate.** Certain samples (e.g., sediment samples, peat material, etc.) may require a more aggressive cleaning process to further digest organic materials. With experience, a technician can learn to anticipate these problem samples. Potassium dichromate is quite effective in furthering the digestion. The potassium dichromate crystals should be added very slowly and cautiously, as additional reactions may occur. **ADDITION OF POTASSIUM DICHROMATE MUST TAKE PLACE IN THE FUME HOOD.**

8. QUALITY ASSURANCE/QUALITY CONTROL

- 8.1. Observed loss of material at any stage of this procedure compromises quantitative samples. Samples which experience such loss should be discarded immediately if

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sufficient material has been retained to allow for a fresh sample. If the compromised sample is the only material available from the original sample, the procedure should be completed, and the nature of spillage or loss should be documented clearly on the “Diatom Slide Preparation Form.”

- 8.2. Diatom frustules are microscopic, generally falling in the fine silt size range; therefore, the possibility for contamination of samples is great. Laboratory rooms where raw or processed samples are handled should be kept as clean as possible. Lab bench surfaces should be kept clean and free of debris. Techniques similar to those used for sterile experiments (bacteriological plating, etc.) should be followed to minimize the risk of cross contamination of samples. Where feasible, disposable pipettes, stirrers, etc. should be used. Where they cannot, they should be rinsed in DW or RO water after each sample, and stored dry.
- 8.3. Samples with live algae should be refrigerated and kept in the dark (live diatoms are capable of continued growth as long as they are receiving light).
- 8.4. New glassware and digestion vessel liners should be washed and/or rinsed prior to use. Used glassware should be vigorously scrubbed, washed with a detergent, and rinsed at least three times with DW or RO to prevent contamination. Previously used digestion vessel liners should be washed with a detergent and soft brush (beware of abrasives, however) and rinsed at least three times with DW or RO. (Explanatory note: at times tap water, because of algal blooms and use of diatomaceous earth filters, may contain diatoms.) All equipment should be stored dry to prevent growth of algae or fungi.

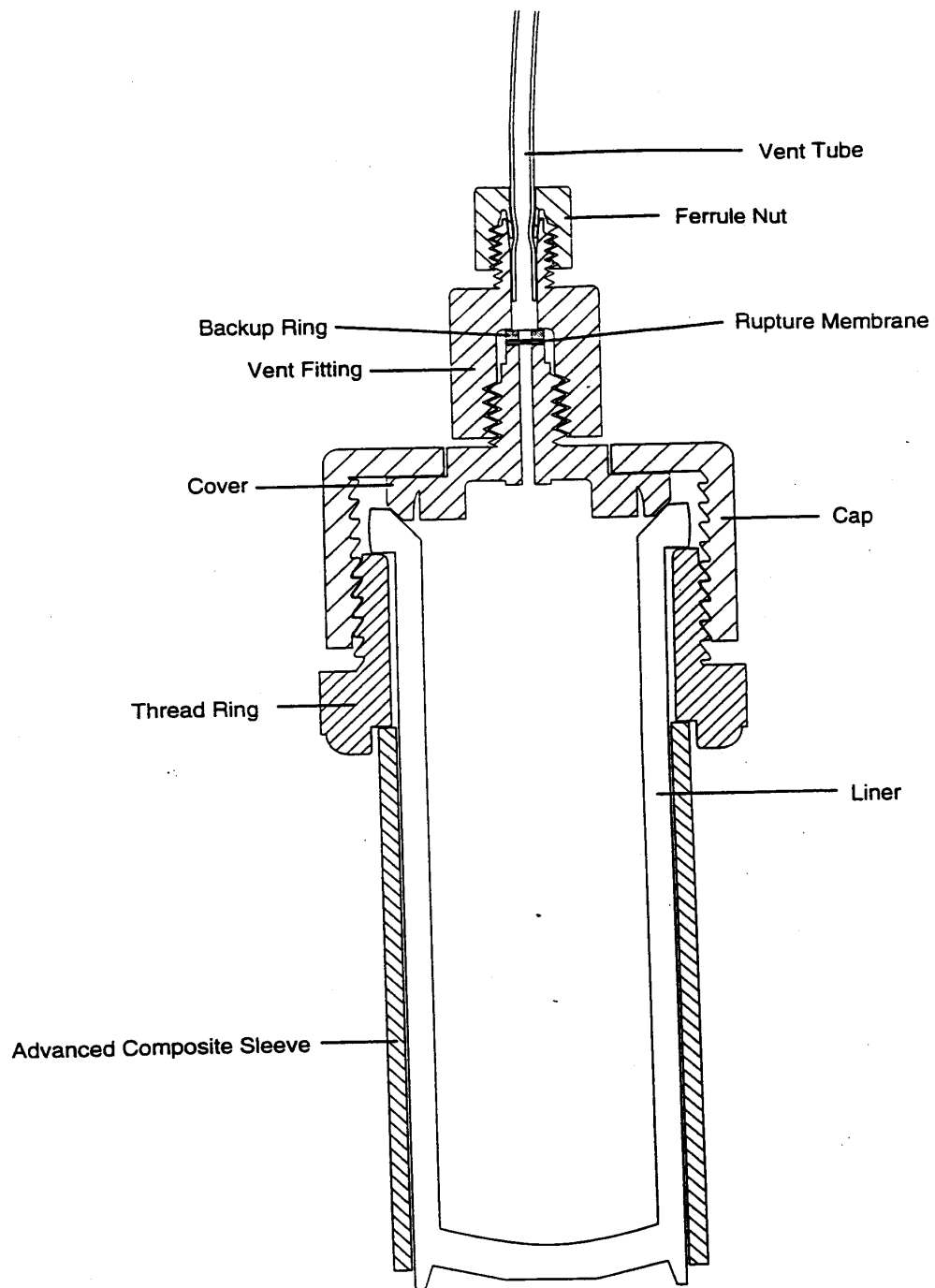


Figure 1. Standard Advanced Composite Vessel (cross section).

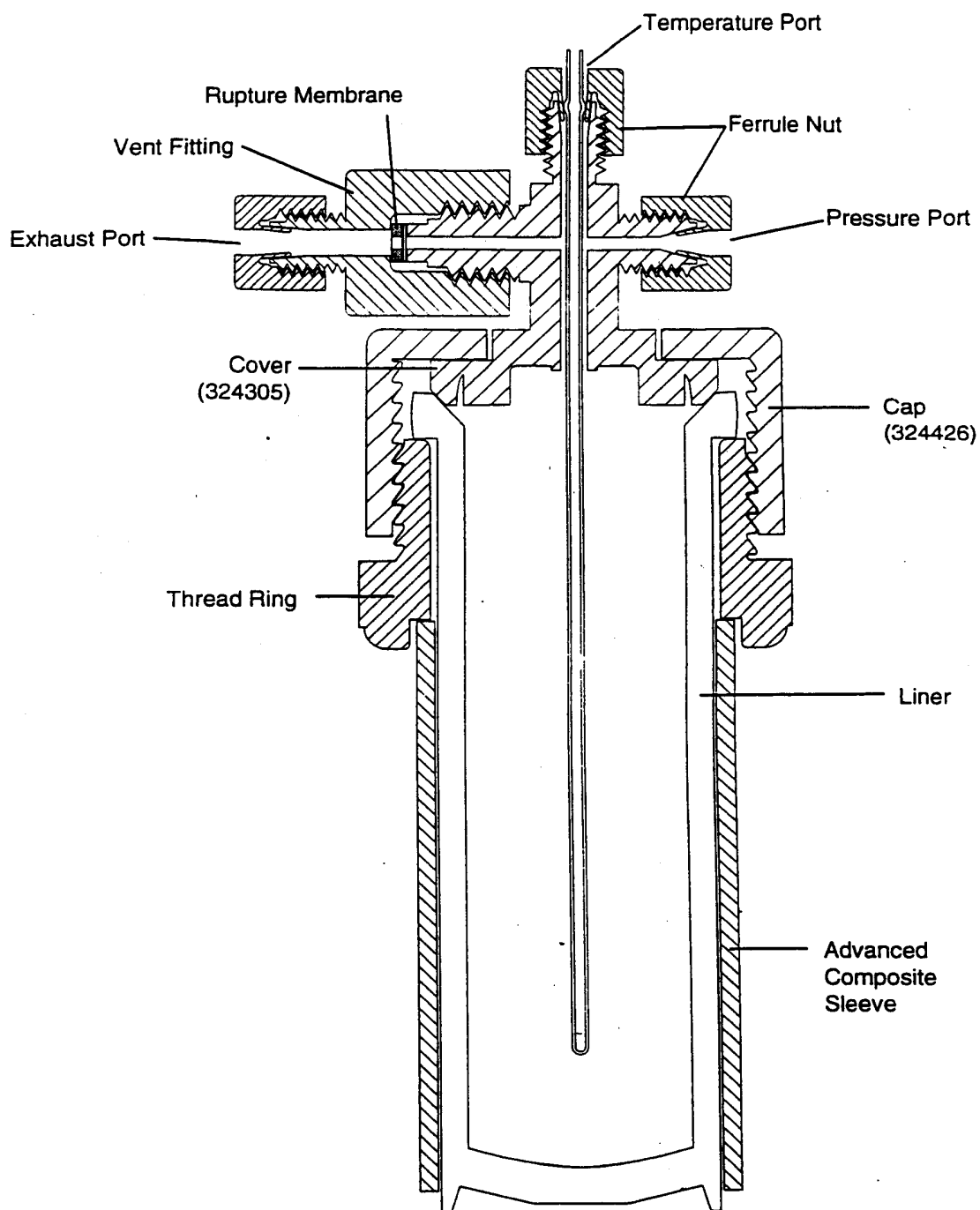


Figure 2. Advanced Composite Vessel for Pressure and Temperature Control.

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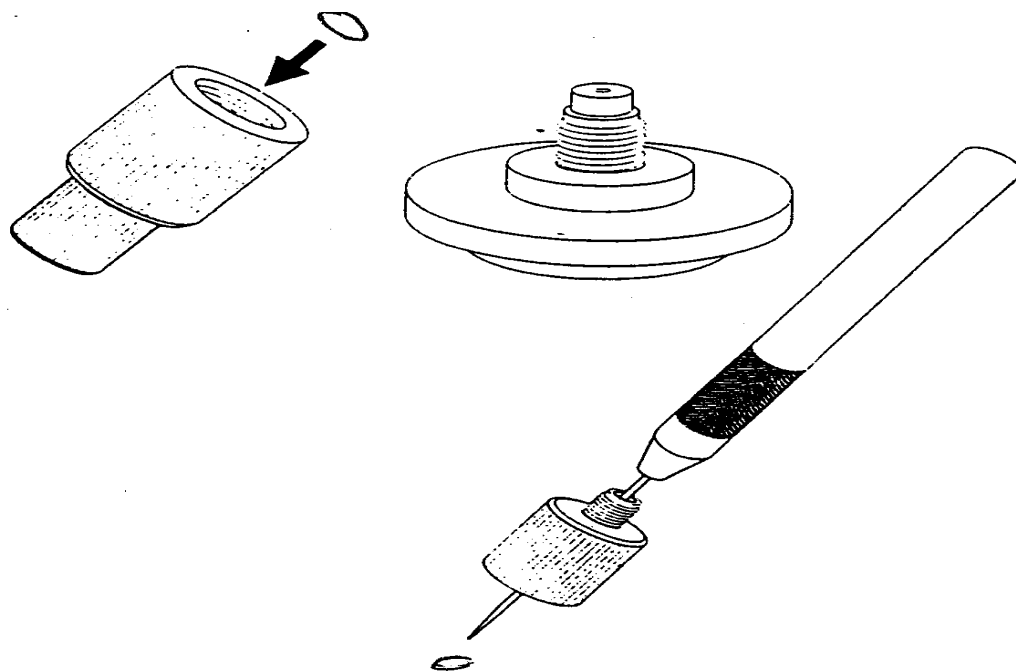


Figure 3. Installation and Removal of Rupture Membrane.

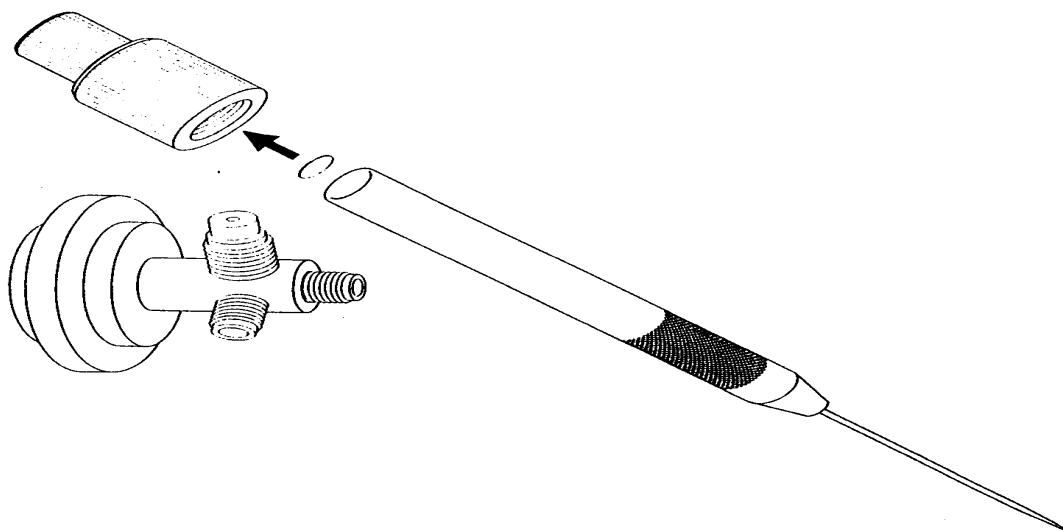


Figure 4. Installation of Rupture Membrane in Vent Fitting of Advanced Composite Vessel with Temperature/Pressure Control Cover.

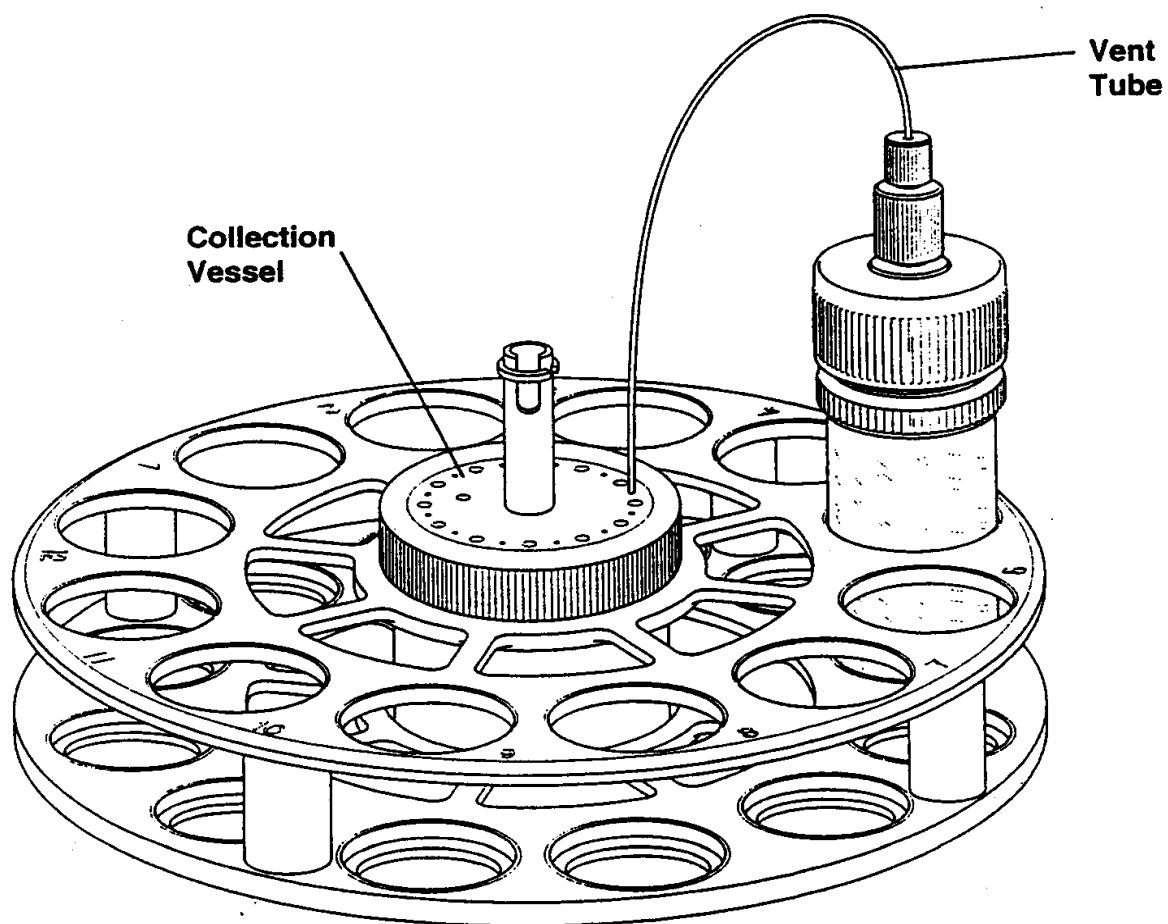


Figure 5. Routing of Pressure Sensing Line and Fiber Optic Temperature Probe.

Procedure No. P-13-49

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-49
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**PREPARATION OF DIATOM SLIDES USING NAPHRAX™
MOUNTING MEDIUM**

Prepared By: Frank Acker, Benjamin Russell and Eduardo Morales

Preparation of Diatom Slides Using Naphrax™ Mounting Medium

Frank Acker, Benjamin Russell and Eduardo Morales

1. PURPOSE

- 1.1. Accurate identification and enumeration of diatoms requires mounting of cleaned material between a microscope slide and cover slip in a medium with a refractive index near that of glass, so that the features of diatom frustules or valves are clearly visible at high magnification. Naphrax™, a commercially-available toluene-based mounting medium with high refractive index, is currently used at the ANSP. This protocol details the steps necessary to produce high-quality diatom mounts from cleaned diatom material. This technique produces 'permanent' mounts, preserving the diatom specimens over many decades, at least.

2. SCOPE

- 2.1. Procedures described in this protocol include the dilution and dispersion of cleaned material onto glass cover slips, the mounting of cover slips onto glass microscope slides using Naphrax™ mounting medium, and the labeling of permanent mounts suitable for inclusion in the ANSP Diatom Herbarium.
- 2.2. This procedure applies to personnel involved with the preparation of diatom slides.

3. REFERENCES

- 3.1. American Public Health Association, American Water Works Association, Water Environment Federation (APHA, AWWA, WEF). 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed.
- 3.2. Patrick, R. and C. Reimer. 1967. The Diatoms of the United States. Vol. 1. Monograph No. 13, Academy of Natural Sciences of Philadelphia, 688 pp.
- 3.3. PCER, ANSP. 1990. Laboratory Safety Manual.
- 3.4. U.S. Environmental Protection Agency. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-73-001. US. EPA Office of Research and Development. Cincinnati, OH.

4. SAFETY PRECAUTIONS

- 4.1. Personnel should be familiar with the information given in Reference 3.4.
- 4.2. Naphrax™ should be considered a hazardous substance because it contains toluene, an organic solvent. Toluene volatilizes readily when heated. For this reason, heating of Naphrax™ should only be performed under a positive-draw chemical hood. Personnel should wear safety glasses and protective hand wear when working with liquid Naphrax™

at room temperature, when heating Naphrax™ in a hood, or when in contact with solidified Naphrax™ toward the final stages of slide preparation.

- 4.3. Hot plate temperatures required for this procedure are high enough to cause severe burning of exposed skin. Use extreme care when manipulating slides on the hot plate and at times when working close to the hot plate.

5. APPARATUS/EQUIPMENT

- 5.1. Corning ceramic-top hot plate with temperature control.
- 5.2. Positive-draw chemical hood.
- 5.3. Aluminum drying plate (25.5 x 20.0 x 0.5 cm, solid aluminum; lines forming 48 squares, each 3.2 cm on a side, are etched on the surface. Each square is etched with an identifying number).
- 5.4. Glass microscope slides (1 x 3 inches; 2.5 x 7.5 cm)
- 5.5. Glass cover slips (18 mm x 18 mm) - No. 1 thickness, stored in covered glass jar filled with 100 % ethanol.
- 5.6. Naphrax™ mounting medium.
- 5.7. Diamond scribe.
- 5.8. Disposable plastic pipettes.
- 5.9. Adjustable pipettor (0 - 250 µl); adjustable pipettor (200 - 1000 µl).
- 5.10. Pipette tips for adjustable pipettors.
- 5.11. Round-style tooth picks.
- 5.12. Forceps.
- 5.13. Polished, rounded wooden splints.
- 5.14. Wash bottle filled with distilled (DW) or reverse osmosis (RO) water
- 5.15. Single-edged razor blades.
- 5.16. Ethanol, 70%.
- 5.17. Acetone.
- 5.18. Kimwipe® tissues.
- 5.19. ANSP slide labels.
- 5.20. Wax (the kind commonly used for candle making and canning foods).

6. METHODS

- 6.1. **Estimate amount of cleaned diatom material to deposit on coverslip.**

- 6.1.1. Starting with cleaned material contained within 20-ml glass vials, estimate the volume of suspended material that will need to be deposited (“dripped”) on a cover slip to produce a slide of the appropriate cell density. The ideal density to be achieved on the final mount is somewhat subjective and is based on the amount of debris in the sample, the preferences of the slide analyst and the way in which the slide is to be used. Generally, between 5 and 10 diatom specimens should be present in a single high power microscope field (1000X). To make the estimate, shake the cleaned material to ensure a homogeneous dispersion of cells within the 20-ml vial. Immediately open the vial and withdraw either a 25- or 50- μ l subsample using the 0- to 250 μ l adjustable pipettor. Place the subsample on a slide and cover it with an 18 x 18 mm cover slip. Then observe this preparation under a compound microscope at 50X magnification. Look at a number of fields and observe the density of cells. Then calculate the amount of material that would need to be dripped so that the density of cells seen at this magnification would be approximately 30 to 40 per field. This estimate is referred to as the “drip count” (the amount of cleaned material to be placed on a cover slip). Accuracy of estimates improves with experience. In many cases, analysts will request that both a “heavy” slide (~40 cells/field) and a “light” slide (~30 cells/field) be made. Record the “drip count” estimates on the “Diatom Lab - Slide Preparation Notes” form (Figure 1). When slidemaking is complete, record the estimates and final amounts dripped on the “Diatom Slide Preparation Form” (See Figure 2, Protocol P-13-48). Also note observations of interfering materials (sand, silt, etc.) on this form.
- 6.1.2. In some cases, the number of diatoms in a sample is very sparse. This is usually because diatoms were rare in the habitats sampled, or the sample bottles contain a small amount of material. In these cases, additional procedures are required to either make a satisfactory slide for analysis or to determine that analysis of a sample is not practical. Follow these procedures if more than about 900 μ l (this is the maximum amount that a coverslip can “hold” beneath it) would need to be dripped onto a coverslip to meet the above criteria.
 - 6.1.2.1. If a satisfactory slide could be made by increasing the concentration of cleaned diatom material by two to five times, then do this by using a micropipettor to remove the required amount water from the vial of material after it has been allowed to settle for at least eight hours. Record the concentration factor on the “Diatom Slide Preparation Form.”
 - 6.1.2.2. If a concentration of cleaned material greater than two to five times is required, then re-subsample the original sample (Protocol No. P-13-48). Take a subsample of a size sufficient to prepare satisfactory slides. Use all of the remaining sample only if absolutely necessary. Digest the subsample and prepare a new vial of cleaned material (Protocol P-13-42). Repeat procedure 6.1.1, above. If the concentration of cleaned material is still not sufficient, concentrate it, as described above. If still too dilute, combine the two vials of

cleaned subsample materials. Record steps and volumes, and final concentration factor, on the “Diatom Slide Preparation Form.”

6.1.2.3. If, after following the steps above to concentrate the cleaned material, the density of diatoms on a cover slip still does not meet the criteria of 30 to 40 cells per field at 400 - 450 X magnification, then proceed to make the densest slide possible and take it to a diatom analyst to evaluate (the Phycology Section Diatom Taxonomy Coordinator at the ANSP). The analyst will make a determination of whether it is practical to analyze the sample. They will quickly scan the slide in its entirety under 100X magnification, and estimate the total number of individuals on the slide. Then they will make their determination, taking into account the density of diatoms, evidence of dissolution, and amount of debris (silt, clay, broken remains of diatoms and other siliceous organisms) that would make it difficult to identify specimens accurately. As a general guideline, if accurate identifications are possible, and at least 100 specimens could be counted within four hours, they should determine that the slide be analyzed; otherwise it should not. If the diatom analyst determines that the slide should not be counted, inform the Phycology Section Project Manager immediately. They will call the NAWQA study unit biologist that submitted the samples to inform them of the problem. Only under very special circumstances will extraordinary measures (e.g., very long counting time) be taken to analyze a slide. Record results of the diatom analyst's determination and rationale on the “Diatom Slide Preparation Form.”

6.1.2.4. When doing their evaluation of a slide with few diatoms, as described in the step above, a diatom analyst may occasionally see evidence suggesting that a sample contains lightly silicified diatoms that may not have survived the digestion process. In these rare instances, they may suggest that a “burn mount” be made to determine whether diatoms did exist in the original sample. (This is one reason why a small portion of the initial sample should always be saved, even for phytoplankton.) The burn mount procedure was used extensively to create slides for diatom analysis before the introduction of methods incorporating acids for the digestion of organic material. Even though this method does not rid sample material entirely of organic debris, diatoms on the slide can at least be identified as diatoms. For this method, follow the EPA (1973) procedure. Briefly, a known portion of the untreated sample is dripped onto a coverslip and allowed to dry at room temperature. When the sample is dry, it is placed onto a hot plate and left for about 30 min at ca. 570 °C. The coverslip is mounted according to procedure 6.3, below.

After it is prepared, have the burn mount slide examined by a diatom analyst. They will determine if diatoms are present and whether analysis of the slide is warranted. Slides prepared using the burn mount method can not be counted if too much organic material remains on the slide. This is because it is not possible to make accurate taxonomic identifications. Generally, burn mounts are used only as a last resort, and to confirm that weakly silicified diatoms are

not present in the sample. Record information on all burn mount attempts, successful or unsuccessful, on the “Diatom Slide Preparation Form.” Include at least date, name of preparer, volume of subsample used, and whether diatoms were observed.

- 6.2. **Deposit cleaned material on coverslip.** Use forceps to remove single 18 x 18-mm cover slips from the ethanol storage container, and wipe each clean carefully with a Kimwipe. Place each cover slip on a marked space of the aluminum drying plate. Be sure the aluminum drying plate is clean and dry to avoid cross-contamination. If the intended drip count is less than 600 µl, drip a small amount of distilled water onto the cover slip with a disposable pipette, sufficient to form a thin layer of water over the entire cover slip. Agitate the sample vial to a uniform dispersion and use the adjustable pipettor to quickly withdraw the required amount from near the central portion of the sample. Eject this material smoothly and carefully onto the layer of distilled water already on the slip. By alternately drawing material up into the pipette and ejecting it, a homogeneous suspension is achieved on the cover slip. In the case where more than ~600 µl of original sample is required, the addition of distilled water is not necessary, and the sample can be ejected and mixed directly on the cover slip. In both cases, care should be taken to ensure that the suspension covers the entire surface of the cover slip, including the extreme edges of the corners. Should the cover slip overflow, discard the cover slip, and repeat the procedure with a freshly cleaned cover slip. Discard the pipette tip when finished with each sample.

Once the aluminum drying plate is loaded with cover slip preparations, the plate should remain undisturbed until the cover slips are dry. At this point, drying of the slips can proceed at room temperature (a period of several hours will be required), or gentle heat (warm to the touch only) may be applied to hasten evaporation (a light bulb placed 15 - 30 cm over the drying plate is one option). Once completely dry, put the aluminum plate with cover slips on the hot plate that has been preheated to 250 to 300°F. Leave for 3 to 5 minutes. This procedure ensures that nearly all water is driven from the material on the cover slips and helps assure that the diatom frustules will adhere to the surface of the glass. Remove the aluminum plate from the hotplate and inspect the cover slips. If the pattern of diatoms distributed on any of the cover slips is not even and smooth, they should be re-dripped. If cover slip distributions seem unsatisfactory after repeated attempts, consult an algal analyst.

- 6.3. **Mount coverslip on microscope slide.**

- 6.3.1. Using a diamond scribe, etch microscope slides with Sample ID, Subsample ID and Slide Replicate ID (e.g., GS029231 DT1 a).
- 6.3.2. Mount coverslip on slide.

THE FOLLOWING STEPS MUST BE PERFORMED IN A POSITIVE-DRAW FUME HOOD!

Using a rounded wooden splint or disposable pipette, transfer a small amount of Naphrax (volume equivalent to ~2 to 4 drops of water) to the central portion of the etched side of the microscope slide. Using a rounded wooden toothpick, distribute the Naphrax over an area approximately equivalent to the size of the cover slip. Then remove the appropriate cover slip from the aluminum plate with forceps, being careful to handle the cover slip only at the extreme corners. Invert the slip and place it gently on the Naphrax covered portion of the slide. Then place the slide (cover slip up) on the hotplate and apply gentle heat until the evolution of bubbles resulting from the evaporation of the toluene solvent first occurs, and then significantly diminishes. Remove the slide from the hot plate, and, using the rounded toothpicks, gently position the cover slip and press it to form a uniform, thin layer of Naphrax™ beneath the entire cover slip. Make sure that the edges of the cover slip are brought parallel to the edges of the microscope slide. Care must be taken at this stage not to press so hard as to damage or dislodge the diatoms or cause warping of the cover slip. As this procedure is taking place, the Naphrax™ is “setting up” (becoming hard), and the ability to move the cover slip will diminish rapidly. At this point, set aside the mount to finish cooling.

- 6.3.3. Use a single-edge razor blade to carefully trim any excess Naphrax™ which has been squeezed out from beneath the cover slip. Great care must be taken to avoid “lifting” the cover slip by inadvertently allowing the edge of the blade to move between the cover slip and the microscope slide. Once most of the excess Naphrax has been removed and discarded, and while still working under the hood, place the mount in successive baths of acetone, and then ethanol for no more than 10 or 15 seconds each. Finally, wipe the mount clean with a Kimwipe tissue.
- 6.4. **Add paper label to slides.** Either before or after slides have been analyzed, depending on project requirements, prepare paper labels and attach them to the mounts following the exact specifications and examples contained in Appendix 1. The standard labels produced by the Phycology Section include those for diatometer projects, surveys (hand collections), general projects (miscellaneous types), and the USGS NAWQA program. Contractors often submit slides with only etched labels; paper labels are added by Phycology Section staff.
- 6.5. **Enter data from the “Diatom Slide Preparation Form”.** Enter data directly into the following fields of the “Slide Information” table in the PHYCLGY database: Sample ID, Diatom Subsample ID, Slide Replicate ID, Vol Cleaned Material, D/C Factor, uL dripped, Settled By (Worker ID), Mounted By (Worker ID), and Date Diatom Slide Completed.
- 6.6. **Assemble forms and transmit slides.** Put slides in plastic slide boxes; label each with name of project and subproject, Subproject ID, Box _ of _, date (month/year) box prepared, and name or initials of preparer. Sign and date the “Diatom Slide Preparation Form” and the “Diatom Lab - Slide Preparation Notes” form and put them in the “Diatom Analysis” folder. Print a “Diatom Slide Analysis Form” for use by the diatom analyst and add it to the “Diatom Analysis” folder also. Create

this form using the “Diatom Analysis Form(…)” report in the PHYCLGY database. When slides are completed, transmit them and associated forms to the Phycology Section Project Manager, or inform them that slides are prepared.

- 6.7. **Preserve and store cleaned material.** After slides are analyzed according to the appropriate protocol, and no additional slides need to be made, process the vials containing the remaining acid-cleaned material for long-term storage. Working under the fume hood, add two - four drops of 100% buffered formalin to each vial (some contractors use alcohol as a preservative instead). Tightly cap the vials and seal them by immersing the top 1/3 of the vial in melted wax. Then transfer the vials to the appropriate storage cabinet in the Phycology Section for long-term storage. Be sure that the cabinet and shelves on which they are stored are properly labeled with the study unit year and subproject ID. See the Phycology Section Project Manager for assistance.

7. QUALITY ASSURANCE/QUALITY CONTROL

- 7.1. This procedure was developed in the laboratories of the ANSP and has been used for the preparation of several thousand slides. Naphrax is produced under quality control conditions specifically for the purpose of high resolution slides (Northern Biological Supplies of Islip Great Britain). Naphrax mounts have proven to be stable over long periods (there are 25 plus year mounts in the ANSP Herbarium) and has been the mounting medium of choice of European investigations for over 40 years. Before it's production was halted in 1993, Hyrax was the most widely used commercially-available mounting medium and was used at the ANSP for many years before the switch to Naphrax.
- 7.2. It should be understood that, given the microscopic size and large numbers of diatoms which are transferred from the cleaned material vials to the finished mount, there are a number of steps where contamination of the samples is possible. Laboratory rooms where raw or processed samples are handled should be kept as clean as possible. Laboratory bench surfaces should be kept clean and free of debris at all times. Techniques similar to those used for sterile experiments (bacteriological plating, etc.) should be followed to minimize the risk of cross-contamination of samples. All equipment coming into contact with sample material should be rinsed in DW or RO water at least three times. Disposable pipettes should be used when possible.
- 7.3. The distribution of specimens on the final mounted cover slips should represent the samples contained within the cleaned material vials. The degree to which this is true depends on how well the cleaned material is dispersed prior to sub-sample withdrawal, and how evenly the withdrawn material is dispersed on the cover slip. Great care should be taken to ensure that these two steps are completed properly.
- 7.4. For certain critical applications, the project protocol may call for duplicate slide sets to test for variation in quantitative data introduced by this procedure.

Diatom Lab - Slide Preparation Notes

Phycology Section

Patrick Center for Environmental Research

The Academy of Natural Sciences

1	1	2	2	3	3	4	4
5	5	6	6	7	7	8	8
9	9	10	10	11	11	12	12
13	13	14	14	15	15	16	16
17	17	18	18	19	19	20	20
21	21	22	22	23	23	24	24

Notes:

Dripped By:

Date Dripped:

Project Number & Name:

Figure 1. Diatom Lab - Slide Preparation Notes form.

APPENDIX 1

SLIDE LABEL FORMAT (ANSP DIATOM HERBARIUM)

Listed below are formats for slides labels for diatometer projects, surveys and special projects. Labels are 1 inch (2.54 cm) square and can accommodate 10 lines with 15 characters or spaces per line. Abbreviations should be used when necessary and clear.

I. Diatometer Projects

Line #1: State abbreviation, county name (or abbreviation)
e.g. SC, Allend. Co.

Line #2: Waterbody
e.g. Savannah River

Line #3: Station and substation code (diatometer code)
e.g. Sta: 1RC

Line #4: Installation date (exposure date)
e.g. exp: VIII-12-87

Line #5: Removal date
e.g. rem: VIII-26-87

Line #6: (nothing; assumes no particular collector)

Line #7: Project name
e.g. Sav Diatom. #35

Line #8: sides scrapped, microliters dripped
e.g. 6 sides 1500 μ l

Line #9: (nothing; reserved)

Line #10 preprinted Acad Nat Sci Philadelphia or ANS Phila.

Example:

SC, Allend. Co.
Savannah River
Sta: 1RC
exp: VIII-12-87
rem: VIII-26-87
Sav. Diatom. #35
6 slides 1500 μ l
ANS Phila.

II. Surveys (Hand Collections)

Line #1: State abbreviation, county name (or abbreviation)
e.g. TX, Victoria Co.

Line #2: Waterbody
e.g. Guadalupe River

Line #3: Station/substation/collection #
e.g. Sta 1L Coll: 19

Line #4 and
Line #5: Microhabitat
e.g. on rocks, gravel and sand

Line #6: Collection date
e.g. X11-10-1987

Line #7: Collector
e.g. Coll: RR Grant

Line #8: Survey name and number
e.g. Sav Cur Sur #96

Line #9: Microliters dripped
e.g. 1500 μ l

Line #10 preprinted Acad Nat Sci Philadelphia or ANS Phila.

Example: TX, Victoria Co.
Guadalupe River
Sta 1 Coll: 19
on rocks, gravel and sand
XII-10-1987
Coll: RR Grant
Sav Cur Sur #96
1500 μ l
ANS Phila.

III. General Projects (Miscellaneous)

Line #1: State abbreviation, county name (or abbreviation)

e.g. PA, Wayne
OH, Clermont

Line #2: Waterbody or installation

e.g. Swago Pond
P&G Art Streams

Line #3: Station and substation or treatment

e.g. Treatment: NPC1
Stream 1, Rep 1

Line #4: Microhabitat (if necessary)

e.g. nutrient pots
glass slides

Line #5: Installation or collection dates (if applicable; exp = exposure date)

e.g. exp: VIII-11-87
VIII-31-87

Line #6: Removal date (if applicable)

e.g. rem: VIII-31-87

Line #7: Collector (if applicable)

e.g. JW Sherman

Line #8 : Project name

e.g. POCONOS 1987-88
1988 P&G
ART. STREAMS

Line #9: Sides scrapped, microliters dripped (if applicable)

e.g. 6 slides 1500 μ l

Line #10 preprinted Acad Nat Sci Philadelphia or ANS Phila.

Examples:

PA, Wayne	OH, Clermont
Swago Pond	P&G Art Streams
Treatment: NPC1	Stream 1 Rep 1
nutrient pots	glass slides
exp: VIII-11-87	VIII-31-87
rem: VIII-31-87	
Coll JW Sherman	1988 P&G
POCONOS 1987-88	Art Streams
6 slides 1500 μ l	
ANS Phila.	ANS Phila.

IV. USGS NAWQA Program

Listed below is the format for slides labels for USGS NAWQA Program. Two labels are generated for each slide. One to be placed to the left of the coverslip and the other to be placed to the right of the coverslip. Abbreviations should be used when necessary and clear.

Left Label:

Line #1: State abbreviation, county name (or abbreviation)
e.g. WI, Milwaukee

Line #2: Waterbody
e.g. Lincoln Cr.

Line #3: Site Location ID
e.g. GS40869415

Line #4: Microhabitat (if necessary)
e.g. nutrient pots

Line #5: Collection dates
e.g. 5/15/95

Line #6: Collector (if applicable)
e.g. B. Scudder

Line #7: USGS NAWQA

Line #8: USGS NAWQA Sample ID
e.g. WMIC0595ARE0001B

Line #9: ANSP Slide ID
e.g. GS004503-DT1-b

Example:

WI, Milwaukee
Lincoln Cr.
GS40869415

5/15/95
B. Scudder
USGS NAWQA
WMIC0595ARE0001B
GS004503-DT1-b

Right Label:

Line #1: ANSP Diatom Herbarium Accession Number
e.g. 100001b

Lines # 2 through #7: Reserved for names of taxa found on the slide (if applicable)

Line #8: Determiner (If applicable)

Line #9: ANSP

ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-117
Rev. 0 (08/00)

**BENTHIC ALGAE AND SEDIMENT CHLOROPHYLL *a* PREPARATION
AND ANALYSIS**

Prepared By: David Velinsky and Jennifer DeAlteris

Benthic Algae Scraping or Sediment Chlorophyll *a* Preparation and Analysis.

1.METHOD

- 1.1. This is a method for the determination of chlorophyll *a* and phaeopigments in sediments or benthic algae (i.e., periphyton).

2. METHOD SUMMARY.

- 2.1. The most useful chemical method for determining the total quantity or biomass of benthic algae (i.e., periphyton) or sediment algae (i.e., microphytobenthos) is to estimate the amount of chlorophyll (as Chlor *a*). This value is used to determine the amount of plant biomass that is present in the sediment. In general, the fluorometric method is 5 to 10 times more sensitive than the spectrophotometric method, but may be less accurate if high concentrations of chlorophyll *b* are present. However, the filter arrangement in the Turner TD-700 yields accurate chlorophyll *a* values. In addition, due to high concentrations of benthic algae, the spectrophotometric method can also be used.

The procedure below outlines the method used to estimate algal biomass. The field collection of samples is described in other operating procedures. Field samples should be stored frozen at all times (< -20°C) prior to analysis.

3. APPARATUS.

- 3.1. Pre-cleaned and tared; 250 ml HDPE bottles or 50 mL centrifuge tubes
- 3.2. Labeling tape
- 3.3. 45°C drying oven
- 3.4. Metal spatula
- 3.5. Analytical Balance capable of weighing to 0.001 g
- 3.6. Distilled Deionized Water (DDW) and wash bottle
- 3.7. Tekmar Tissue homogenizer, Waring Laboratory blender or appropriate mixing system
- 3.8. Sorvall RC-5B refrigerated superspeed centrifuge
- 3.9. 15-ml glass centrifuge tubes and Teflon lined lids
- 3.10. 5% (v/v) HCL and eye dropper
- 3.11. ACS quality acetone diluted with DI water (90% v/v)
- 3.12. Chlorophyll *a* standard free of *b* and *c* forms (Sigma Chemicals)
- 3.13. Fluorometer (Turner Designs TD-700 or equivalent) and proper lamp and filters

4. PROCEDURE.

(Note: In laboratory, all analysis should be performed in a darkened room.)

- 4.1. Take clean 250-ml HDPE bottles or 50-ml centrifuge tubes and rinse each container with some mild detergent and copious amounts of DDW. Air dry or use a low temperature oven (< 40°C). Place marking tape on each bottle and write an ID # on the tape. Weigh

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each bottle, with its cap, to nearest 0.01 g and record the weight on the bottle and in a data sheet.

- 4.2. In the field, place each sample into a pre-weighed bottle (See Collection SOP: D. Charles). Fill each bottle no more than 2/3 full with water and algae, if possible. This sample will be used for total chlorophyll *a* and other parameters, if needed (% organic matter, carbon, nitrogen, etc.). Keep samples at 4°C in the field. If samples cannot be homogenized within 24 hr, freeze samples for storage. It is best to homogenize the samples and remove excess water as soon as possible (within 48 hr).
- 4.3. In the laboratory, keep samples cold. If there are large rocks in the samples, they may be scooped out carefully with a metal spatula prior to grinding. Put sample into clean, DDW-rinsed Warning glass or metal blender. Dependent on the sample, grind and homogenize each sample for less than 1 minute on low. In some cases the samples do not need to be placed into the blender and can be mixed with a simple spatula.
- 4.4. Transfer the ground sample back into the pre-weighed bottle, rinsing any remaining sample from the blender into the bottle with minimal amounts of site water or DDW, if no site water is available.
- 4.5. Keep samples in the dark and cold. Repeat steps 4.3 and 4.4 for six samples (i.e., the rotor head of the centrifuge can hold six samples).
- 4.6. Weigh ground sample bottles so that all are within 0.2 g of each other (add site water or DDW to adjust bottle weights). This is to ensure that there is an even balance in the centrifuge.
- 4.7. Centrifuge samples, using Sorvall RC-5B refrigerated superspeed centrifuge for 20 min at 4°C and 2000 rpm. **Carefully** decant overlying water. Reweigh bottle to the nearest 0.01 g.
- 4.8. Samples may be stored frozen at this point. It is best to analyze samples within 1 month of collection, however there are references and communications that suggests that frozen samples can be stored up to 3 to 6 months.
- 4.9. Chlorophyll *a* Preparation
 - 4.9.1. When ready for analysis, thaw samples if necessary, and homogenize by stirring with a spatula. Take approximately 0.2 g of sample, record the weight to nearest 0.001 g, and place the subsample in a small 15-ml test tube used for chlorophyll. Add 5 ml of 90% acetone. Grind sample gently with a Teflon tissue homogenizer, then rinse the homogenizer with 5 ml of 90% acetone into the centrifuge tube using Pasteur pipettes (i.e., long tipped eye droppers). Total volume should be 10 ml. Cap tube and shake. Place in rack in refrigerator for 48 hr, shaking

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(agitating) twice daily. Each sample is done in duplicate, with every 10th sample in triplicate. (NOTE: For better replication or for other reasons decided upon by the PI, larger sample sizes can be used. This means scaling up the amount of 90% acetone used in larger tubes. Samples must not contain substantial amounts of water as this would dilute the acetone and lower the extraction efficiencies, this is the purpose of the centrifugation.)

- 4.10. After the final agitation, let all sediment settle down to the bottom of the tube (wait about 30 min). Carefully, using Pasteur pipette, take enough sample and place it in the cell and read on fluorometer. (See operating manual for fluorometer). IF SAMPLE READING IS OFF SCALE, (which will happen if there is any green color), then DILUTE sample. As an example, take 100 µl of sample and dilute to 10 ml (100 x dilution), use graduated cylinder (marked to the nearest 0.1 ml). Record fluorescence and dilution.

NOTE: The fluorometer needs to be calibrated every month. (Current information suggests that once every three months may be good as long as the Turner solid standard is stable.) Turner check standards, blanks, and triplicates should be run every 10 samples. Also, samples can be run using a spectrophotometer, without as much dilution.

- 4.11. Add 2 drops of 5% HCl, invert tube three times, wait at least 2 min for reaction to go to near completion and record fluorescence after acid. This yields data for phaeopigment calculations.
- 4.12. Calculate chlorophyll *a* concentrations to µg/g wet weight. By knowing the total mass of wet benthic algae/sediment and surface area of sample, calculate µg Chlor *a*/area or units that are appropriate.

5. QUALITY CONTROL/ASSURANCE OBJECTIVES.

- 5.1. Precision between duplicate samples can be calculated as the RPD and should be within the range of ±20%. Triplicate analysis can have relative standard deviations (RSD = std. dev./mean X 100) of less than 10%. If RPDs or RSDs are higher, evaluate whether the sample can be homogenized. Depending on collection, there could be small rocks or pebbles in the sample that would limit the duplication of the analysis. If the RPD is outside this range, qualify the data as estimated using the letter J.

ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-113
Rev. 0 (6/99)

**DETERMINATION OF DRY WEIGHT AND PERCENT ORGANIC
MATTER FOR SEDIMENTS, TISSUES AND BENTHIC ALGAE**

Prepared By: Paul Kiry, David Velinsky and Anne-Marie Compton

Determination of Dry Weight and Percent Organic Matter for Sediments, Tissues and Benthic Algae

1. METHOD

- 1.1. Gravimetric determination of solid phase dry weight and percent organic matter for sediments, tissue, and benthic algae samples.

2. METHOD SUMMARY

- 2.1. A well mixed sample is weighed into a pre-weighed dish and dried to constant weight at 103-105°C. The dried material is combusted at 550°C for 1 hour and cooled until constant weight for ash weight.

3. APPARATUS

- 3.1. Aluminum pan for dry weight or a pre-labeled porcelain dish for dry weight and ash weight.
- 3.2. High temperature furnace, 550°C
- 3.3. Drying oven, 103-105°C.
- 3.4. Desiccator and fresh desiccant.
- 3.5. Analytical balance, capable of weighing to 0.1 mg.
- 3.6. Forceps and scoop (2).
- 3.7. High temperature insulated-gloves
- 3.8. Long tongs or forceps
- 3.9. Cooling tray
- 3.10. Double deionized water (DDW) in a squeeze bottle.

4. PROCEDURE

- 4.1. Dry Weight at 103-105°C

- 4.1.1. Place a cleaned Al pan in the drying oven at approximately 105°C for one hour. Remove the pan using forceps and place in a desiccator to cool for 1 hour (use similar times throughout). Using forceps place the pan on the balance for weighing. Repeat the drying cycle until a constant weight is obtained (weight loss/gain is less than 0.5 mg). Keep pan in a dry, dust free location for storage.
- 4.1.2. Weigh into the pan a known amount of sediment, tissue or benthic algae, being sure to homogenize the sample prior to transfer.
- 4.1.3. Place the pan+solid material into the drying oven for at least 24 hr. Remove the pan and solid phase material using forceps and place into a desiccator to cool for 1 hour (use similar times throughout). Using forceps place the pan and dried material on the balance and record weight. Repeat the drying cycle until a constant weight is obtained (weight loss/gain is less than 0.5 mg).
- 4.1.4. Calculate the % Solids as the ratio of the dry weight to total wet weight, times 100; subtracting out the weight of the pan:

$$\% \text{ Solids} = \frac{((\text{Dry Weight} + \text{Pan Weight}) - \text{Pan Weight})}{((\text{Wet Weight} + \text{Pan Weight}) - \text{Pan Weight})} \times 100$$

4.2. Percent Organic Matter at 550°C

- 4.2.1. For this procedure, substitute a pre-etched or labeled porcelain dish for the Al pan in Step 4.0.1 The dish should be pre-heated to 550°C for 1 hour and weighed to constant weight.
- 4.2.2. Follow Steps 4.2 to 4.5 to first obtain the dry mass of material.
- 4.2.3. Place the dish with dried material into pre-heated (550°C) furnace for 1 hour.
NOTE: Use high temperature gloved and long tongs to handle dishes. Also, if sample contains high amounts of organic matter heat sample slowly to 550°C to avoid flame combustion.
- 4.2.4. Remove dishes from furnace and place onto cooling tray and allow to cool to room temperature.
- 4.2.5. Carefully wet sample with a small amount of DDW. (This allows the minerals to re-hydrate after the high temperature furnace).
- 4.2.6. Dry samples to constant weight at 103-105°C as outlined above.

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- 4.2.7. Calculate the %Organic Matter as the amount of dry mass of material minus the residual material at 550°C remaining in crucible divided by the total dry mass of material:

Percent Organic Matter (g Residual material/g dry weight) =

$$\frac{(\text{Dry Weight} + \text{Dish Weight}) - (\text{Residual Weight} + \text{Dish Weight})}{(\text{Dry Weight} + \text{Dish Weight}) - \text{Dish Weight}}$$

5. REFERENCES

- 5.1. Standard Methods for the Examination of Water and Wastewater 1995. Edited: A.D. Eaton, L.S. Clesceri and A.E. Greenberg. 19th Edition. Fixed and Volatile Solids (2-57) and Periphyton Sample Analysis (10-32).

3. Analysis protocols

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Procedure No. P-13-65
Rev. 0 (11/02)

**PROCEDURE FOR SEMI-QUANTITATIVE ANALYSIS OF
SOFT ALGAE AND DIATOMS**

Prepared by: Karin Ponader and Diane Winter

Procedures for Semi-quantitative Analysis of Soft Algae and Diatoms

1. PURPOSE:

- 1.1. This protocol describes a semi-quantitative procedure for analyzing the soft algal component of samples to estimate the relative proportions of soft algae and diatoms. As part of a multidisciplinary approach to stream and river research, soft algae samples are collected individually, or split out of diatom samples, to be analyzed for species content and biovolume. These samples usually represent a composite of algal samples collected from natural substrates (e.g., Charles et al., 2000). Samples could be collected from either individual substrate types (e.g., rocks) or from the majority of micro-habitats in a defined sampling zone, reach or station.

2. SCOPE:

- 2.1. This is a semi-quantitative procedure designed to estimate the percentage (or proportion) that each genus/species contributes to the total number of algal cells and the algal biovolume of the sample. This is a two-step method. The first step (Section 6.3) involves identifying the most common genera/species and estimating the relative percentage of each of these in the algal assemblage. In the second step (Section 6.4), the relative percentage that each genus/species contributes to the algal biovolume in the sample is estimated. As this is a semi-quantitative method, cells are not counted or measured, but a general estimate is arrived at which describes the relative proportions of the common genera/species observed in the sample through examination of several transects.
- 2.2. This procedure is applicable for analysis of soft algae in a wide variety of samples where the objective is a semi-quantitative estimate of the relative proportions of algal genera/species contributed by the most common taxa. It is not designed to provide a complete list of taxa present or precise algal abundance or biovolume estimates.
- 2.3. This procedure applies to personnel responsible for taxonomic analysis of soft-algae samples.

3. REFERENCES:

- 3.1. Bahls, L.L., 1993. Periphyton bioassessment methods for Montana streams. Montana Water Quality Bureau, Department of Health and Environmental Science, Helena, MT.
- 3.2. Barbour, M.T., Gerritsen, J., Snyder, B.D. and Stribling, J.B., 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish, Second Edition. EPA 841-B-99-002, Washington, D.C., U.S. Environmental Protection Agency, Office of Water.

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- 3.3. Firth, C. 2002. Counting procedures for algae analysis and measuring of cell biovolumes for calculating biomass percentages. PCER Procedure P-XX-XX. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA.
- 3.4. Charles, D., Winter, D., Hoffman, M. 2000. Field sampling procedures for the New Jersey Algae Indicators project. PCER Procedure P-13-64. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA.
- 3.5. Sprouffs, K., 2002. Subcontractor Soft Algae Database Guide. Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA.

4. DEFINITIONS:

- 4.1. Biomass: the amount of algal biogenic material in a sample by weight.
- 4.2. Biovolume: the amount of algal biogenic material in a sample by volume.
- 4.3. Soft Algae: the non-diatom component of the algae sample.

5. APPARATUS/EQUIPMENT:

- 5.1. Research quality microscope with 10X and 40X objective.
- 5.2. Microscope slides, 75 x 25 mm.
- 5.3. Glass microscope cover slips, rectangular, 22 x 50 mm, #1 thickness.
- 5.4. Plastic pipettes, 5.25 inch.
- 5.5. Pre-printed bench sheets (attached).
- 5.6. Distilled water (algae-free water of any sort).
- 5.7. Computer with Microsoft Access software installed, access to a copy of the Contracted Soft Algae Database created to allow entry of data to larger database system (Sprouffs, 2002), and internet connection for data transferal (if working away from the PCER). The database can be found either in G:\Phycdata\DATABASE\Contracted Soft Algae DB, if at PCER, or will be sent electronically to analyst.

6. METHODS:

- 6.1. Print out bench sheets, see attachment 9.1.

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6.2. Prepare the slide for analysis.

- 6.2.1. Shake each sample to dislodge epiphytic algae and randomize algal cells and colonies. Use scissors or other sharp implements (razor blade, etc.) to cut up any long filamentous algae present in the sample. Immediately after mixing, use a plastic pipette to deliver a portion (2 or 3 drops) of the sample onto a waiting slide. The exact amount of sample analyzed is not as important as is the need to represent the entire algal community. If there are large clumps of filamentous algae, snip off the tip of the pipette to enable their inclusion in the subsample. These large clumps should then be evenly dispersed over the area of the coverslip.
- 6.2.2. Place edge of coverslip at an angle of 45° to the slide surface and next to the sample suspension. Carefully allow the coverslip to fall gently onto the sample suspension.
- 6.2.3. To avoid extensive exposure to formalin fumes, if not working in a hood, have a small fan running during the preparation of the samples.
- 6.2.4. To make sure the density of algal cells in the sample is uniform between the samples, examine the slide at 10X. If too dense, dilute the sample with distilled water, and repeat from step 6.2.1. If too dilute, make sure to report this in the notes of the bench sheet and database.

6.3. Estimate the relative abundance of cell numbers for each genus/species.

- 6.3.1. Scan the entire slide at 10X to estimate the abundance rating based on the relative number of cells for each of the larger soft algal genera/species (see 4.3) in the entire sample. Use the abundance scale below (Table 1) to report relative abundance estimates. Use the abundance rating definitions as a guideline only, if the sample is very sparse, the analyst should use his/her best judgement in assigning abundance rating categories.

Table 1: Abundance rating scale for Section 6.3.

Average Entered in Computer	Estimated % of Cell Observed	Abundance Rating
0.5	0-1	R – (rare) – Only one or two cells observed during entire scan
3	1-5	F – (frequent) - More than one cell is observed, but they appear only sporadically
12.5	5-20	C - (common) - Individual cells appear in several fields of view
30	20-40	A – (abundant) - One or two cells appear in most fields of view
55	40-70	VA – (very abundant) - Multiple cells appear in most fields of view
85	70-100	D – (dominant) - Cells greatly exceed those of other algae in numbers

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- 6.3.2. Switch to 40X to estimate the abundance rating for the smaller algal species, including diatoms. The abundance of all diatoms is to be estimated as a single group. Randomly examine 8 - 10 fields of view, or more if necessary, to obtain a representative estimate of the percentage of the most common genera/species in the sample. Try to take only 30 minutes for both the 10X and 40X examination, not including time spent on species identification.
- 6.3.3. If identification to a species level will be a lengthy process, do not spend excessive time on species identification if the genus/species abundance is rare. In this case, identify only to the genus level (e.g., *Scenedesmus* spp.).
- 6.3.4. Record Abundance Rating abbreviation (e.g., "C") in the appropriate column for each species on the data sheet while counting (see data sheet). The average percentage assigned to each abundance rating is what will be entered in the computer database when the examination is complete (e.g., 12.5 for an abundance rating of "C").
- 6.4. Estimate the percentages of biovolumes of each genus/species.
 - 6.4.1. After abundance ratings are determined, assign biovolume percentages to each genus/species observed. Re-examine the slide to aid in the estimation of the relative proportions of the algae in the slide. Do not assign percentages to species that contribute less than 10% to the total algal biovolume. Group all diatoms together in a single category.
 - 6.4.2. Estimate percentages only of diatoms which contain chloroplast material. Switch to 40X if necessary to make this distinction.
 - 6.4.3. There is no fixed scale for the biovolume percentage estimates, percentages should be assigned based on individual analyst opinion. Do not estimate percentage increments smaller than 5% (e.g., 10, 15, 20, etc.).
 - 6.4.4. This estimation of biovolume should not exceed 10 minutes.
 - 6.4.5. The main objective of this analysis is to record the most common species in the sample. Always list the proportion of diatoms in the assemblage, but do not record the genus/species of other algae which occur as less than 10% of the community.
 - 6.4.6. Record any additional notes on the datasheet, and in the "notes" column in the Contracted Soft Algae database ("Soft algae processing data" Table). These may include the condition of the sample, the condition of the algae, the presence of macrophytes or moss, and other information deemed important. If there are genera/species which occur in <10% of the sample, but might be useful in interpretation of the data, they should be listed in the notes, as well.

7. QUALITY ASSURANCE AND QUALITY CONTROL:

- 7.1. The results of these analyses will be compared with results of analyses from samples collected in different areas. It is important that the level of effort be similar. With the exception of time spent learning new floras, this analysis should be finished within 30-45 minutes and no more than 1 hour. Samples with >90% diatoms can be

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analyzed very quickly. On average, ideally, 10-15 samples should be analyzed per day (7 hrs). If these time limits are being exceeded consistently, it must be reported to the Project Manager and noted on the bench sheets as a protocol deviation.

- 7.2. Minor protocol deviations should be noted on the bench sheets and reported to Project Manger. Any major deviation should be reported to the Project Manager prior to proceeding.
 - 7.3. Minor protocol modifications should be reported to the Project Manager and noted on the Phycology Section's master copy of the protocol. Numerous and major modifications will require a protocol revision and approval by the Patrick Center Quality Assurance Officer.
 - 7.4. Completed electronic database will be e-mailed and paper copy of bench sheets will be sent to PCER upon completion of project samples.
8. DATA ENTRY:
Refer to "Subcontractor Soft Algae Database Guide" (Sprouffske, 2002), which can be found at G:\Phycdata\DATABASE\Contracted Soft Algae DB\Subcontractor Instructions.doc.
9. ATTACHMENTS:
- 9.1. Semi-Quantitative Analysis of Soft Algae and Diatoms Bench sheet (following page).
 - 9.1.1. This method takes into account only the two dimensional area taken up by the algal specimens. Due to time restrictions of this method, the third dimension cannot be taken into account. The authors are aware that the term volume is not appropriate in this case. This method is designed to provide only a rough estimate of biovolume.

Semi-Quantitative Analysis of Soft-Algae and Diatoms					
The Academy of Natural Sciences, PCER, Phycology Section					
Project:					
Sample ID:					
Analysis Date:					
Analyst:					
	Estimated			Estimated	
	Cell #	Bio vol.		Cell #	Bio vol.
Taxon Name:			Taxon Name:		
			Bacillariophyta (Diatoms)		
Notes:					

Procedure No. P-13-65

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-13-39
Rev. 0 (11/02)

**ANALYSIS OF DIATOMS ON MICROSCOPE SLIDES PREPARED
FROM USGS NAWQA PROGRAM ALGAE SAMPLES**

Prepared by: Todd Clason, Frank Acker, Eduardo Morales, and Lont Marr

Analysis of Diatoms on Microscope Slides Prepared From USGS NAWQA Program Algae Samples

1. PURPOSE

- 1.1. The USGS NAWQA program collects four kinds of algae samples analyzed by the Phycology Section of the Patrick Center for Environmental Research, ANSP. These include Richest Targeted Habitat (RTH), Depositional Targeted Habitat (DTH), Qualitative Multiple Habitat (QMH), and phytoplankton samples (Porter et al., 1993). This protocol describes procedures for analyzing diatoms on microscope slides made from all four types of NAWQA algae samples.
- 1.2. The purpose of RTH and DTH sample analysis is to estimate the proportion of diatom taxa found in a count of 600 valves (one-half of an individual diatom cell). Results are later combined with those from analysis of the soft-algae component of the same sample (Protocol P-13-63) to provide data on algal densities (as cells per cm² of sampling surface) and amount of algal biovolume (μm³ per cm² of sampling surface) at a sampling site.
- 1.3. The purpose of QMH sample analysis is to identify as many taxa present in the sample as possible, to provide an accurate and uniform estimate of algal taxa richness in a stream reach. An underlying assumption is that although all algal taxa present in a sample (or on a slide prepared from a sample) will not be identified, most species will be found during a reasonable search. If that effort is consistent among taxonomists, results from analyses of samples and slides will be comparable among analysts and contract laboratories” (Porter 1994). Unlike an RTH and DTH count, the number of diatoms to be counted is not fixed. Instead, the analyst scans the slide until the rate at which new species are encountered, per 100 specimens observed, drops below a defined number, or a time limit is reached.
- 1.4. The purpose of phytoplankton sample analysis is similar to that of RTH and DTH samples, except that the quantitative results are expressed in number of cells per volume of water. Phytoplankton samples are collected from the water column, using various sampling techniques and collection devices.

2. SCOPE

- 2.1. This protocol covers the identification and enumeration of diatom taxa mounted on microscope slides. Two alternative procedures are described for recording data: 1) use of the “Tabulator” program (Cotter 2002), and 2) writing on bench sheets followed by data entry directly into database tables or through use of the “DtmCnt” program. As of summer 2001, all NAWQA diatom analysts use the “Tabulator” program. For this reason, the primary methods described here pertain to analyses made using “Tabulator.” The only section relating to the “bench sheet” approach lists the data fields that must be entered into the database for each analysis. Most of the instructions for using “Tabulator” are in the User’s Guide (Cotter 2002). Some of those instructions are summarized here to provide an

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overview of the program and to help clarify how it is used in the process of analyzing NAWQA samples. Procedures not included in the “Tabulator” manual are described here.

- 2.2. This procedure is applicable to the analysis of diatoms in algae samples collected by the RTH, DTH, QMH, and phytoplankton sampling protocols of the USGS NAWQA program.
- 2.3. Personnel responsible for these procedures include diatom analysts and data entry personnel.
- 2.4. In March 2002, the previous version of this Protocol (RTH and DTH samples only) was merged with Protocol P-13-61, which described procedures for analysis of NAWQA QMH samples.

3. REFERENCES

- 3.1. Cotter, P. 2002. Tabulator Installation and User’s Guide. Version 3.51. ANSP, PCER.
- 3.2. PCER, ANSP 1999. Diatom Cleaning by Nitric Acid Digestion with a Microwave Apparatus. Protocol No. P-13-42.
- 3.3. PCER, ANSP . 1999. Analysis of Soft Algae and Enumeration of Total Number of Diatoms in USGS NAWQA Program Quantitative Targeted-habitat (RTH and DTH) Samples. Protocol P-13-63
- 3.4. PCER, ANSP 1999. Preparation of Diatom Slides Using Naphrax™ Mounting Medium. Protocol No. P-13-49.
- 3.5. Porter, S.D. 1994. Amendment to Guidance, Procedures, and Specifications for Processing NAWQA Algal Samples by Contract Laboratories. Email contract reference of 10/19/1994 from Stephen Porter to Allison Brigham of the USGS, Ann St. Armand of Phycotech, Don Charles and Frank Acker of the Academy of Natural Sciences.
- 3.6. 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, NC.
- 3.7. United States Geological Survey, National Water-Quality Assessment Program. 1997. Protocols for Processing NAWQA Algal Samples. Draft Manuscript. February 1997.

4. APPARATUS/EQUIPMENT

4.1. Compound microscope:

- 4.1.1. Oil immersion objective (100x) with a numerical aperture of at least 1.3;
- 4.1.2. Eyepieces of 10-15x; DIC (differential interference contrast) or bright field condenser;
- 4.1.3. Diamond scribe mounted on microscope’s objective stage;
- 4.1.4. High intensity light source.

4.2. Desktop computer (located at microscope if “Tabulator” program is used):

- 4.2.1. Pentium II or higher processor;
- 4.2.2. Software: “Tabulator” or “DtmCnt” programs by Patrick Cotter (MS Visual Basic);
- 4.2.3. Network connection to ANSP Phycology Section databases (ANSP staff only).

5. METHODS

5.1. Diatom counts.

- 5.1.1. Review the “Diatom Slide Preparation Form” and the “Diatom Slide Analysis Form” (Figure 1) contained in the “Diatom Analysis” folder and transmitted with the diatom slides from the Diatom Preparation Lab. The “Diatom Slide Analysis Form” lists sample information for each slide it accompanies, and provides space next to each listed slide to initial and date when a count is finished. It also serves as a chain-of-custody record; it must be signed by the person delivering the slides and the person receiving them. Make sure that the slides correspond with the entries on the form. Note and resolve any discrepancies.
- 5.1.2. Scan slides at low to medium magnification (100X to 450X) to confirm that diatoms are evenly distributed on the coverslip, and are at a density appropriate for efficient counting. At high magnification (1000X), there should be between 5-10 diatoms per field. If there are problems with dispersion or density that would compromise the quality and accuracy of the analysis, discuss these with Diatom Preparation Lab personnel and have new slides made. Avoid counting diatoms in any disrupted areas of the mount, particularly edges that have optical aberrations. If diatoms on the slides are very sparse, refer to procedures in Protocol No. P-13-49 for handling low-density samples. Always save any count data generated for a sample, even if the number of valves or frustules is low (e.g., <100).
- 5.1.3. Because slides may need to be recounted for QA/QC purposes, it is very important to clearly demarcate the areas of a slide scanned during a count. After the preliminary slide examination, secure the slide in the mechanical stage and use the microscope’s diamond scribe to etch a horizontal or vertical line (depending on personal preference) on the coverslip to mark the edge of the first row to be counted. Rows are narrow rectangular areas (strips) of the slide adjacent to the scribed line, with width equal to the field of view. Start rows far enough from the coverslip edge to avoid optical distortion, and end them near the opposite coverslip edge where diatoms are no longer clearly visible (Figure 1). Locate a starting point near one end of the etched line and make a circle with the scribe. This denotes the starting point of the count. During the count, etch a circle around the last field counted in the first row and at the beginning and end of all other rows. Always check to make sure that etching is clearly visible so that circles and lines can be located easily by others.

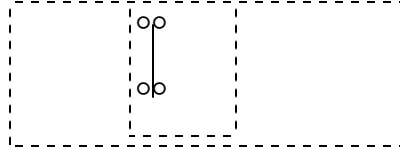


Figure 1

- 5.1.4. When the line and first field are etched on the coverslip, and the first field is focused under oil immersion, begin using the “Tabulator” program, following the instructions in the manual; some steps are summarized below. After opening the program, the first screen encountered is the Count Information page. Click the “New” button along the bottom edge of the page. Most fields will automatically fill with default information if this is not the first slide in the Subproject to be counted; otherwise data must be added. Enter data in the fields in the “Slide” box at the top of the form. Click the “Verify Slide” button to reconfirm that the slide information is in the database. Fill in the other fields in the form, including “Frustules or Valves” and “Count Type.” For RTH and DTH samples, choose “Valves” and then “600 valves (300 cells).” For QMH samples, choose “Frustules” and then “NAWQA Qualitative (diatoms).” (Note that the selection in the “Frustules or Valves” box determines the choices available in the “Count Type” box). Click the “Save” button.
- 5.1.5. After the preliminary information is recorded, click the “Count Now” button. Several small text boxes are displayed to confirm data entry, and then the main Tabulator page appears. Before counting can proceed, select a taxa list from the bottom right “Choose List” box. The Tabulator manual describes how to create new lists and add new taxa to existing lists.
- 5.1.6. Before beginning the count, click the “Note” box in the central portion of the Tabulator window and record the start circle coordinates (numbers on the microscope stage). Coordinates of the first (and last) field of each row should be recorded immediately after they are scribed using the following format: “Row 1 x35.2, y87; y95; Row 2” etc. The x coordinate should only be recorded once for each row.
- 5.1.7. As the count commences, enter taxa observed using the discrete three-digit codes established during the taxa list building process. Enter codes with the numeric keypad on the computer keyboard. Record multiple examples of a single taxa either with code: “322 +10 enter,” for example; or by repeated hits of the enter key “322 enter, enter, etc.” Taxa may also be subtracted by typing the taxon code, followed by a minus sign and the number to be subtracted: “322 –1 enter,” for example. The program will signal an alert when the count total, as established by the count type, is reached (quantitative counts only).

On average, analysis of a slide should take approximately two hours; in no case should it exceed four hours. This does not include time spent learning new taxa when analyzing the first few samples in a new study unit.

- 5.1.7.1. RTH and DTH analysis. Count 600 valves. Count all partial valves that are more than 50% of the valve or that contain unique features such as recognizable

central areas or distinct ends. Count all valves and fragments that extend at least halfway into the field of view.

- 5.1.7.2. QMH analysis. The stopping rule for QMH samples is: “Taxa found on semi-permanent slides are examined and identified in intervals (groups) of 100 frustules or valves. When examining the first interval of 100 individuals, determine if any taxon constitutes 40 percent or more of the total. Such predominant taxa should not be tallied in subsequent intervals. A minimum of 10 intervals (1000 individuals) is to be examined. Continue scanning intervals until two consecutive intervals have been completed in which two or fewer new taxa are encountered. It is unnecessary to scan more than 50 intervals (5000 individuals) per sample. Record the number of intervals scanned on the laboratory data sheet” (Porter 1994).

Use the following procedure for counting QMH samples with the “Tabulator” program. Count the first interval as if it were a regular RTH or DTH sample. That is, record in “Tabulator” the occurrence of each frustule viewed under the microscope. Once the first 100 hundred frustules have been counted, generate an on-screen report for the count by going to the File menu on the Tabulator screen and selecting Print Count. From this report, determine if any taxa in the first interval equaled or exceeded a relative abundance of 40%. Exclude these taxa from the remainder of the analysis. Close the on-screen report. Starting with the second interval, keep track of the number of frustules by using a hand counter. Record occurrence of a new species (species not encountered in the first interval) in Tabulator only once, the first time it is encountered. Each time an interval is finished, type “r” and then hit enter on the keyboard to proceed with the next interval. A message will appear: “Are you sure you want to end this interval?.” Hit yes. “End Row ?” (where “?” is the number of the row just completed), will then appear in the “Count Entries” window. If the 9th and 10th intervals contain two or fewer new taxa, then stop the analysis at the end of the 10th interval. Otherwise, continue the analysis until two consecutive intervals are found for which no more than two new species are recorded, up to a maximum of 50 intervals (5000 frustules).

- 5.1.8. When the count is finished, return to the Count Information page to complete the boxes “Date Count Finished,” “Scan Length,” and “Hours To Complete”. Then return to the Tabulator window and print a “Count Report” and check it carefully for errors. Make adjustments, if necessary, print a final copy, sign it, and put it in the “Diatom Analysis” folder. In Tabulator, select “Save Count” from the “File” menu in the top left of the Tabulator window. This saves the count to the underlying database. If the count data are not saved before exiting Tabulator, the information will be retained by the program but not added to a database. To save the data if this occurs, reopen the “Tabulator” program, enter the required information about the sample, click “Count Now” to get to the Tabulator window, and select “Save Count” from the “File Menu.”

Put initials and date on the “Diatom Slide Analysis” form next to the entry for the slide just counted. Return it and any other related forms to the “Diatom Analysis” folder. Clean slides of immersion oil with alcohol. When finished analyzing all slides in a subproject, give the slides and “Diatom Analysis” folder to the Phycology Section Project Manager.

5.2. Biovolume measurements.

5.2.1. NAWQA sample analysis requires biovolume measurements for each taxon occurring in abundance of 5% or more in any one sample in a study unit. Criteria for determining how many measurements to make of each taxon for each NAWQA study unit changed slightly from the beginning of algal analyses (1995). The basic rule, as originally specified by NAWQA, was to make 15 sets of measurements. As the number of measurements for taxa accumulated, however, the criterion was changed. Since 1999, only 5 additional sets of measurements are required for taxa in new study units if the range of those 5 sets falls within the range of all previous measurements from other study units. If the ranges do not overlap, make a full 15 measurements for the taxon. Biovolume measurements can be made during the routine process of counting slides or after all slides for a Subproject have been counted. It is likely that criteria for selecting specimens to measure will evolve as the number of measurements for common taxa accumulates.

5.2.2. Use the form “frmBiovolumeVerification” in the PHYCLGY database to determine which taxa occur in abundance of 5% or more in samples in a Subproject, and therefore must be measured. The form also shows the number of measurements that are already entered in the database, and minimum, maximum and average biovolumes. Print the results. To find which slides contain the most specimens of the taxa to be measured, use the query “qryfind=>5% taxa.” Print the results. Both of the above printouts should be included in the Diatom Analysis folder.

5.2.3. Use the Biovolume Calculation feature of Tabulator as a convenient means for calculating and entering biovolume data directly into the NADED database (ANSP staff) or the “Tabulator” back-end database (subcontractors). The BioVol program can also be used. It is essentially a stand-alone version of the “Biovolume Calculation” feature in “Tabulator.” It is located in G:\Phycdata\VBAppInstalls\BioVol.

Choose the first microscope slide with taxa to be measured, open the “Tabulator” program, and enter the “Slide ID.” Select the “Find Counts” and “Count Now” buttons, and make the choices necessary to get to the Tabulator screen. Select “Biovolume Calculation” from the Documentation menu at the top of the Tabulator screen. The fields labeled Sample ID, Subsample ID, Slide Replicate ID, Microscope, Lens, and Conversion Factor are automatically filled with information for the slide you entered. Drop-down boxes can be used to modify any of this information if needed. The Microscope ID, Lens ID and Conversion Factor fields are linked. As soon as a given Microscope and Lens are selected from the drop-down box, a conversion factor for that microscope is shown in the Conversion Factor field. Since conversion factors are already stored in a database table

accessible to the “Tabulator” program, and they are used in the calculation of biovolumes, it is extremely important NOT to make any conversion in the measurements before entering them in the required field of the Biovolume Calculations form. Simply enter readings from your ocular scale directly and as they are!

Enter the NADED Taxon ID for the specimen being measured. The taxon name field will fill-in automatically. Then select the correct shape for the taxon. If it is already filled-in, make sure it is correct. Consult the Biovolume Measurements table in NADED as a reference source for assigning shapes. The table contains shape codes that have been assigned to taxa in the past. The shape specifies a specific formula to be used to calculate biovolume. The measurement fields that must be filled-in for that shape will appear on the form. Be sure to enter data for all required dimensions.

The number of measurements made for a taxon are shown in the field labeled “# of Measurements this session”. If measurements must be corrected, click the “Datasheet” button at the bottom of the form and make changes in the appropriate record. After all measurements for a taxon have been entered, press the “New taxon” button at the bottom left of the form to begin the process with a different taxon. When ready to go to a new slide to make measurements, enter new data in the “Slide” box and follow same steps described above.

The Biovolume Summary window is a useful feature for keeping track of measurements and to check that all have been made for a study unit. It is also useful for comparing measurements for a taxon with all others made for that taxon. It is accessed through the “Edit” menu on the top left of the Biovolume Calculation window. Select a subproject in the central menu, click the “Diatom” button to the right, and all taxa requiring biovolume measurements are displayed. Single taxa can be selected and double clicked to display biovolume measurements for that taxon over all subprojects. This is helpful for determining whether averages of current measurements fit in the ranges determined for that taxon in other study units. Again, reviewing data at this level can prevent significant errors.

5.3. Specimen documentation.

5.3.1. Requirements for documenting diatom species vary with subproject. In general, circle new, unknown, unusual and outstanding diatom specimens with a diamond scribe and image them photographically or with a digital device. This allows comparison with reference specimens and facilitates examination by specialists. Use the following two features, accessible from the Tabulate screen, to assist with documentation.

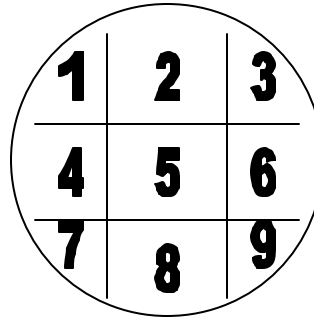
5.3.2. Circle specimens. Click the “Circle” button in the “Tabulation” box in the Tabulator window to activate the New Circle on Slide window. Values that appear in the fields for Taxon name, Microscope, Date, and Circler default [??] from the Tabulator screen. Enter the Circle Number, Horizontal and Vertical Coordinates (from microscope stage), and both Cover Slip Sector (1-16) and Circle Sector (1-9) (see illustrations). These all help document the circle and specimen location. Record extra information

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concerning the documentation in the “Note” box, if necessary. You can click the “Datasheet” button to review records for existing circles. Make sure circles on coverslip are clearly visible.

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

Cover Slip Sector



Circle Sector

- 5.3.3 Circle specimens. Click the “Circle” button in the “Tabulation” box in the Tabulator window to activate the New Circle on Slide window. Values that appear in the fields for Taxon name, Microscope, Date, and Circler default [??] from the Tabulator screen. Enter the Circle Number, Horizontal and Vertical Coordinates (from microscope stage), and both Cover Slip Sector (1-16) and Circle Sector (1-9) (see illustrations). These all help document the circle and specimen location. Record extra information concerning the documentation in the “Note” box, if necessary. You can click the “Datasheet” button to review records for existing circles. Make sure circles on coverslip are clearly visible

Image specimens. Click the “Image” button in the “Tabulation” box in the Tabulator window to activate the Images window. All data in fields in this window automatically default to those in the Tabulator window, including the name of the last taxon counted. If the taxon is not the one you want to document, choose a different name from the drop down box of the same field.

Fill in values in fields under the four tabs.

“Subject” – Taxon name and measurements

Add information in the “Length,” “Width/Diameter,” and “Striae Density” fields, making sure that the measurements are expressed in microns. If your ocular scale is not 1:1 you must make the necessary conversions. The boxes “Quality” and “Public?” can be left untouched since this is information that will be added by Academy personnel reviewing the image before it is made available on the Phycology Section's web site. We are currently not using the “Caption” field and it can be left blank. Add notes referring to any characteristic of the taxon being imaged, or any taxonomic problems that you may have had with it during sample analysis, to the “Notes” field.

“Who, where, when” – Person taking image, location, image device, etc.

Enter the location from which you are working and the “Image Device” you are using for capturing the image. In the “People” box fill the fields labeled “Determiner,” “Imager” and “Adder” with the proper information. Most of these fields, except for “Image Device” will be automatically filled in with the same data that were entered in the fields in the “Count Information” screen.

“ANSP Sample” –sample identification information

Fields are filled in automatically.

Digital images must be taken following the steps and recommendations given in the Taxonomic Guidelines document.

When all information in the "Images" screen is complete, including in the tabbed boxes, press the "Save Record" button located on the top right portion. This will save all data in the NADED database. It will also assign the next available number, which will appear in the Image ID field, in the upper left hand corner. Record this number for future reference. At the time the image data are saved, the identification number (e.g. IM000027) that is assigned is automatically recorded in the “DigitalImage” table of the ALGAEIMAGE database in a field called “ImageID”. A second field called “ImageFileName” will be filled-in at the same time. It contains the identification number with the extension “.png” added (e.g. IM000027.png), which corresponds to the file format used by ANSP to store image files.

Open the imaging program you are using (e.g., Photoshop v 5.5) and edit the image as desired. Save the image in the “Originals” folder located in G:\Phycdata\DATABASE\Images\images. Name the file the Image ID recorded previously.

5.4. **Bench sheets.** Enter data recorded on bench sheets directly into the following fields in the specified ANSP PHYCLGY database tables or by using the “DtmCnt” program. As a quality control measure, data should be entered by someone other than the analyst. The analyst should then review the entered data to verify that they were entered correctly. The “DtmCnt” program is in G:\Phycdata\VBAppInstalls\DtmCnt.

5.4.1. Table “Diatom Count Information” has several fields that must be entered (mandatory), some that should be entered if data available (optional), some that will be added later (verification) and several that should be skipped (not applicable):

5.4.2. Sample identifiers are mandatory: **Sample ID**, **SubSample Replicate ID**, **Slide Replicate**, and **Count Replicate ID**.

5.4.3. **Count Type** is mandatory and is “17” for RTH, DTH and phytoplankton samples, and “33” for QMH samples.

5.4.4. **Taxonomy ID** is mandatory and can be looked up in the “Taxonomy Number” table.

5.4.5. **Frustules? (OrValves)** is mandatory. It is “No” for RTH, DTH, and phytoplankton samples and “Yes” for QMH samples.

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- 5.4.6. **Worker ID** is mandatory, is the ID of the diatom analyst and can be looked up in the “Worker Name” table.
- 5.4.7. **Worker Address ID** is mandatory and can be looked up in the “Worker Address” table.
- 5.4.8. **Date Count Started** is optional.
- 5.4.9. **Date Count Finished** is mandatory.
- 5.4.10. **Date Count Verified** is for verification.
- 5.4.11. **Total Time** is mandatory, and refers to the time necessary for the count.
- 5.4.12. **Verifier Worker ID** is for verification and refers to the Worker ID of the person who verifies that the entered count data represents the actual data from the count.
- 5.4.13. **Source Data Form** is mandatory and can be looked up in the “Source Data Form” table.
- 5.4.14. **Diatom Analysis Form ID** is optional and refers to the code for form used to track the diatom analysis procedure.
- 5.4.15. **Diatom Count Footnote** is not applicable.
- 5.4.16. **Number Counted** is mandatory for RTH, DTH, and PP samples and should be near “600”. For QMH samples it is optional, and refers to the number of frustules scanned during the procedure.
- 5.4.17. **Corresponding H₂O Sample** is not applicable.
- 5.4.18. **Validated** is for verification.
- 5.4.19. **Taxa Notes** is mandatory.
- 5.4.20. **Microscope ID** is mandatory and can be looked up in the “Microscopes” table.
- 5.4.21. **Lense ID** is mandatory and can be looked up in the “Microscope Lenses” table.
- 5.4.22. **Magnification Changer** is mandatory and refers to the amount of magnification from auxiliary lenses (enter 1 if a magnification changer was not used).
- 5.4.23. **Scan Length** is mandatory and refers to the total length of the scan (in mm) during the analysis.
- 5.5. For each diatom species encountered, create a record in the “Diatom Count” table for RTH, DTH and phytoplankton samples, and in the “Diatom Count Qualitative” table for QMH samples. Add data to the following fields:
 - 5.5.1. Sample Identifiers as in section 5.4.2: **Sample ID**, **Subsample ID**, **Slide Replicate ID** and **Count Replicate ID**.
 - 5.5.2. **TaxonID** is the NADED number for the observed taxon.
 - 5.5.3. **NumberCounted** is the number of valves enumerated for RTH and DTH samples; for QMH samples it is the number of frustules observed in the 1st 100-frustule interval.
 - 5.5.4. **NumberCells** is the same as NumberCounted for diatom analyses. This field is not used for QMH samples; leave blank.

5.5.5. **TaxaNote** is “Yes” or “No” depending whether there was a taxa note concerning this taxon.

6. **QUALITY ASSURANCE/QUALITY CONTROL**

- 6.1. Sample and slide quality can affect the outcome of these procedures. Minor deviations that do not affect the area scanned or number of specimens observed should be described on bench sheets or in the Note portion (click the “Note” button) of the “Tabulator” program. Other deviations should be discussed with the Phycology Section Project Manager for inclusion in the project QA/QC notes.
- 6.2. This protocol will be carried out under the general provisions of section 5.4. of ANSP, PCER (2000): “Algal Research and Ecological Synthesis for the USGS National Water Quality Assessment (NAWQA) Program. Draft Quality Assurance Project Plan.” According to this plan, “A total of 10% of the samples collected from each study unit will be analyzed for quality control. There will be two types of QA/QC analyses: a re-count of a diatom slide (taxa harmonization count or THC) and a complete re-processing and re-count of the chosen QA/QC sample (replicate subsample count or RSC). The THC’s will be performed on diatom samples only while the RSC’s will be performed on both diatom and non-diatom samples.” Quantitative comparisons among counts are based largely on Jaccard’s Index and Percent Similarity.