Protocol P-13-48

Subsampling Procedures for USGS NAWQA Program Periphyton Samples

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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 U.S. Geological Survey’s (USGS) National Water-Quality Assessment Program (NAWQA): Richest Targeted Habitat (RTH), Depositional Targeted Habitat (DTH) and Qualitative Multihabitat (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.

2. 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 algal 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.

3. REFERENCES


4. 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.

5. 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-µm mesh).

5.11. Screening apparatus.

5.12. Large plastic disposable weighing boats.

5.13. Analytical balance, capacity to 500 g, 0.1 g accuracy.


5.15. 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 IDs for the set of samples 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 Sample Volume/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 to 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 (microblender, 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
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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, without the maceration step. If there are visible macro forms, selectively add a portion of these to the subsample(s). Prepare 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 μm) 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. 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 P-13-58). 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.

8. **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

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Data Entered By: ____________________________  ___/___/___
Confirmed By: _____________________________  ___/___/___

**Figure 1.** NAWQA Sample Volume/Subsampling Form.
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Remnants of Original Samples Transmitted By: ___________________________ Date: __/__/____ To (location): ___________________________
**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.