Protocol P-13-52

Analysis of USGS NAWQA Program Phytoplankton Samples

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1. PURPOSE

1.1. The U.S. Geological Survey's (USGS) National Water-Quality Assessment Program (NAWQA) samples phytoplanktonic algae by collecting whole-water samples (Porter et al. 1993, Moulton et al. 2002). This protocol describes quantitative procedures for analyzing the soft-algal component of phytoplankton and counting the total number of diatoms.

1.2. This procedure is quantitative and designed to provide data on algal densities (as cells per ml) and amount of algal biovolume (μm^3 per ml) at a sampling site. These data can be compared with data from other sampling sites in the NAWQA program throughout the United States. A similar protocol (P-13-39) describes the procedures for analyzing the diatom component of phytoplankton samples.

2. SCOPE

2.1. This protocol is applicable to the analysis of the soft-algal component of whole-water phytoplankton samples collected by the USGS NAWQA program. It includes procedures for identification (to lowest possible taxon level) and enumeration of algal species, taking measurements of the dimensions of some species for biovolume determinations, and recording of all data.

2.2. Personnel responsible for these procedures include sample preparers, phytoplankton analysts and those involved with data entry.

2.3. Two methods for identifying and counting phytoplanktonic algae are described: 1) using an inverted microscope and a modified Utermöhl sedimentation technique (Hasle 1978), and 2) using Palmer-Maloney counting cells.

3. REFERENCES

3.1. Hasle, G.R. 1978. The inverted-microscope method. Chapter 5.2.1 in Phytoplankton Manual. A. Sournia, ed. United Nations Educational, Scientific and Cultural Organization. Paris. 337 pp.

3.2. Moulton, S.R., II, J.G. Kennen, R.M. Goldstein, J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities in the National Water-Quality Assessment program, U.S. Geological Survey Open-File Report 02-150. In Press.

3.3. Palmer, C.M. and T.E. Maloney. 1954. A new counting slide for nannoplankton. American Society of Limnology and Oceanography Special Publication Number 21. 6 pp.

3.4. PCER, ANSP. 2002. Analysis of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples. Protocol No. P-13-39.

3.5. PCER, ANSP. 2002. Preparation of Algal Samples for Analysis Using Palmer-Maloney Cells. Protocol P-13-50.

3.6. PCER, ANSP. 2002. Subsampling Procedures for USGS NAWQA Program Periphyton Samples. Protocol P-13-48.

3.7. 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. U.S. Geological Survey Open-File Report 93-409, Raleigh, NC [39 pp] http//water.usgs.gov/nawqa/nawqa_home.html.

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

3.9. Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-73-001. National Environmental Research Center, Office of Research & Development, U. S. Environmental Protection Agency. Cincinnati, OH.

4. DEFINITIONS

4.1. **Aliquot.** Is defined as a portion of a liquid sample or subsample.

4.2. **Fraction.** During algal analysis, an aliquot of the soft-algae subsample is used in a counting chamber (use of Palmer-Maloney and Utermöhl chambers are described here). Prior to analysis, the subsample may be concentrated or diluted forming additional solutions from which an aliquot can be taken. We have adopted the term “fraction” to identify the specific solutions that can be analyzed in counting chambers. We identify the original soft-algae subsample as fraction “a;” subsequent solutions, in their own containers, are identified as “b,” “c,” etc.

4.3. **Natural Counting Unit.** Each natural grouping of algae (i.e., each individual filament, colony, or isolated cell) is defined as a natural counting unit. Diatoms are an exception; each diatom cell is always considered a natural counting unit, even if attached to other cells. The main purpose of using 'natural counting units' is to prevent a colonial or filamentous form from dominating a count. It also facilitates the counting of algal forms which have linked cells that may be hard to distinguish.

5. APPARATUS/EQUIPMENT

5.1. Compound microscope with 10-15x, 40-50x and 90-100x objectives. Objectives are mounted below the stage for the inverted microscope method.

5.2. Settling chambers with 10-ml settling tubes. There are several basic varieties of the Utermöhl sedimentation chambers including: (1) the tubular variety (e.g., HydroBios Tubular Plankton Chamber) consisting of a threaded, fitted base with a round cover slip (#1, 27.5 mm diameter) base plate, and (2) the combined plate chamber variety (e.g., PhycoTech Utermöhl Counting Chamber) consisting of a plexiglass base unit with a round cover slip (#1, 27.5 or 25.1 mm) base plate and thick glass (0.2 mm) cover plate.

5.3. Pre-printed laboratory bench sheets including “USGS NAWQA Palmer-Maloney Fraction Preparation Bench Sheet” (See Protocol No. P-13-50), “Phytoplankton Community

Composition Bench Sheet (USGS NAWQA Program)" (Figure 1), and "Algal Biovolume Measurements" form (see Figure 2, Protocol P-13-63).

5.4. 20-ml vials with screw top caps.

5.5. Mechanical tabulator, 8 to 10 positions.

5.6. Palmer-Maloney counting cells with ceramic chamber, chamber depth of 0.4 mm and volume of 0.1 ml.

5.7. Glass microscope cover slips, rectangular, 22 x 50mm, #1 thickness.

5.8. Pasteur pipettes, 5.25 inch.

5.9. Rose Bengal dye, dissolved in 90% acetone.

6. METHODS

6.1. **Choose Analysis Method.** Both analysis methods, inverted microscope and Palmer-Maloney counting cell, result in similar counts when used correctly. The inverted scope is better when the original sample volume is limited (i.e., less than 400 ml) because high numbers of cells are needed for the Palmer-Maloney counts. Detritus, a problem with both techniques, is especially troublesome for the inverted microscope method.

6.2. **Pre-Concentrate Subsamples.** For both techniques, the original sample should usually be concentrated prior to settling in the Utermöhl chamber or adding to a Palmer-Maloney cell. This initial concentration should be approximately 5-10 times the original whole-water sample, leaving about 20 ml of concentrate for analysis. Samples are concentrated by settling and decanting (settle for at least 2 days) or by centrifugation (1000 g for 20 min). It is important to measure and record the original and final volumes, before and after concentration. In the USGS NAWQA Program, this step is usually performed during the subsampling procedure, using the "NAWQA Sample Volume/SubSample Form" (see Protocol P-13-48). This concentrated sample is then divided into at least two subsamples – one for soft-algae or phytoplankton analysis and one for diatom analysis. Phytoplankton analysis using Palmer-Maloney counting cells involves the preparation of "fractions" prior to analysis (Protocol P-13-50). The aliquots placed in the Utermöhl chamber are considered the "fractions," and volume, dilution or concentration data are included in the "Palmer-Maloney Fractions" table (accessible via the PHYCLGY database).

6.3. **Prepare Palmer-Maloney Counting Cell.**

6.3.1. Spread a small drop of Rose Bengal solution on the base of the chamber of a clean Palmer-Maloney counting cell and let dry.

6.3.2. Place a rectangular cover slip (#1 thickness, 22 x 50 mm) at 45° to the counting cell, covering about 1/3 of the chamber, but not across the center of the cell.

6.3.3. Thoroughly mix the Palmer-Maloney fraction and draw it into an elongated Pasteur pipette (5.25 inch). Quickly add the fraction drop-wise into the center of the chamber. When the surface tension starts to draw the cover slip across the chamber, adjust the sides of the cover slip so that the ends of the chamber are covered and the cover slip hangs over both sides of the ceramic portion of the counting cell. Then add glycerin to the area where the cover slip hangs over the ceramic portion. This seals the cover slip to the counting cell temporarily; without excess heat or vibration, the counting cell can be used for a week or more.

6.4. **Prepare Utermöhl Sedimentation Chamber.**

6.4.1. Attach a glass cover glass to the bottom of an Utermöhl sedimentation chamber. For tubular varieties of settling chambers, seal a cover glass to the threaded end of the tube and screw the tube into the base assembly. Assemble the plate chamber type of settling chambers by sealing a cover glass on the bottom of the base, locking it into place with the metal ring, and sealing the cylinder on top of the base. Use a light amount of vacuum grease to seal the cover glasses and cylinders. It is critical that the cover glass be clean and grease-free.

6.4.2. Homogenize the concentrated samples by repeatedly inverting the sample bottle. Place a 10-ml aliquot of the sample into the assembled settling chamber.

6.4.3. Let the sample settle for at least 8 hours.

6.4.4. For the plate chamber type of Utermöhl chamber, drain the volumetric cylinder by sliding over the drainage hole. Slide the cover plate over the chamber without allowing air bubbles to form. Analysis should proceed within a few hours of removing the cylinder.

6.5. **Choose to count random fields or along transects.** Both methods (inverted microscope and Palmer-Maloney counting cell) involve counting phytoplankton cells in a chamber, by counting either random fields or along transects. Choose one of the following.

6.5.1. Determine random fields: Using a high dry microscope objective (40-45x objective, 400-450x total system magnification), identify and enumerate algae in selected, random fields. From each Palmer-Maloney counting cell or Utermöhl chamber, enumerate between 8 and 50 fields; use a second counting cell or sedimentation chamber, if necessary. Choose a random starting place in the upper left-hand quadrant of the counting cell and approximate the number of fields that must be analyzed (300 natural units need to be counted with a minimum of 8 and maximum of 100 random fields). Develop a pattern that allows for equal probability of landing in any area of the cell or chamber with the exception of the edges and the center. A maximum pattern with 50 fields is made by having a grid of 8 x 8, subtracting 3 or 4 fields in either direction of the center.

6.5.2. Determine transects: Using a high dry microscope objective (40-45x objective, 400-450x total system magnification) with a calibrated stage, identify and enumerate algae along transects, either horizontally or vertically across the chamber of the Palmer-Maloney cell or Utermöhl plate chamber. Without looking into the microscope, choose a location near the left edge in the upper third of the chamber (if vertical transects are analyzed, choose a location near the top edge in the left third of the chamber). Make a transect by moving only the horizontal stage control (or vertical control for vertical transects) a measured distance. Develop a pattern for the transects that will avoid the center and edges of the chamber. A second Palmer-Maloney cell or Utermöhl chamber can be used, if necessary (300 natural units need to be counted with a minimum of one complete transect).

6.6. **Enumerate 300 natural algal units.**

6.6.1. Using the pattern developed above (section *6.5.*), move the microscope stage to a new position in the pattern. Make all movements of the microscope stage without looking through the objectives.

6.6.2. Identify and enumerate all algal forms in the field of view: Enumerate algal forms using natural counting units. Natural counting units are defined as one for each colony, filament, diatom cell (regardless if colonial or filamentous) or unicell. With the exception of diatoms, identify algal forms to the lowest possible taxonomic level. Differentiate diatoms to the lowest practical taxonomic level. This will usually be genus, but use of categories like naviculoid, cymbelloid, centric, nitzschoid is appropriate.

6.6.3. Categorize diatoms as either “living” or “dead” at the time of collection, and quantify them separately. If there is any protoplast material in the frustule (usually stained reddish by the Rose Bengal), the diatom is considered to have been living when collected.

6.6.4. Count the number of algal cells comprising each multicellular counting unit.

6.6.5. Tabulate the data on a bench sheet or mechanical tabulator.

6.6.6. Repeat steps *6.6.1.*, *6.6.2.* and *6.6.4.* until 300 natural algal units have been enumerated. Count only “living” diatoms as part of the required 300 natural algal units.

6.6.7. Add and record the tallies of each taxon on the bench sheet. Record the number of cells for multicellular counting units in parenthesis beside the tally of natural counting units. Group all diatoms into one category – undifferentiated diatoms.

6.6.8. Record the number of fields or total length of the transect for the area that was observed.

6.7. **Enumerate larger, rarer taxa.** There is an additional, optional procedure that can be used for samples with low concentrations (less than five natural counting units) of large cells or colonies (maximum dimension greater than 100 µm). Using a low-power objective (10-15x), scan 20 fields or 4 transects. Count the larger, rarer taxa (as defined above). Enumerate as natural units and estimate the number of cells in each. Record the counts of each of the taxa on the bench sheets, noting the scan area (i.e., total area for the 20 microscope fields or 4 transects). Multiply the number of larger, rarer taxa by the ratio of the total area scanned in the regular count to the area scanned in this count. Record that number as the total count for that taxon.

6.8. **Measure cell biovolumes.** For each group of samples, measure the dimensions of the taxa that contribute most to sample biovolume.

6.8.1. Determine the taxa that need biovolume measurements by listing all the species in the samples collected in a NAWQA study unit that have accounted for 5% or more of a sample count (i.e., 15 or more natural units of the 300 natural units enumerated).

6.8.2. For each taxon requiring biovolume measurements, select a simple geometric figure matching the shape of the taxon as best as possible, and determine the dimensions that must be measured (see the “Shapes” table in the NADEDdat database). Record this information on an "Algal Biovolume Measurements" bench sheet (See Figure 2, Protocol P-13-63), one per taxon.

6.8.3. Measure and record the dimensions of at least five specimens. If these measurements are not in the range of previous measurements, measure additional specimens until 15 specimens have been measured from the study unit. No more than five specimens should be measured from a single sample.

6.9. **Enter data.** Enter data recorded on the bench sheets into the following three tables of the PHYCLGY database.

6.9.1. Table "Non Diatom Count Information." There is one record for each analysis. Enter the following fields for each record:

6.9.1.1. **Sample ID, SubSampleID, Replicate ID, Palmer-Maloney Fraction ID** and **Count Replicate ID** describe the specific sample and fraction used.

6.9.1.2. **Count Type** = "37" for "Inverted Microscope Proportional (phytoplankton)" and "38" for Palmer-Maloney Proportional (phytoplankton)" counts.

6.9.1.3. **Worker ID** and **Worker Address ID** are codes for the analyst. These are listed on the bench sheets.

6.9.1.4. **Date Count Finished, Bench Sheet ID, Total Time, Microscope ID** and **Lens ID**, are found on the bench sheet and are mandatory for each analysis.

6.9.1.5. **Palmer-Maloney Field Volume** is found on the bench sheet and is mandatory for analyses using Palmer-Maloney cells and where algae were enumerated in fields (as opposed to enumerating along transects).

6.9.1.6. **Number Fields** is found on the bench sheet and is mandatory for analyses where algae were enumerated in fields (as opposed to enumerating along transects).

6.9.1.7. **VolumeScanned** is mandatory for analyses using Palmer-Maloney cells. For analyses enumerating fields, it is calculated by multiplying the Number of Fields by the Palmer-Maloney Field Volume. For analyses where phytoplankton were enumerated along transects, the VolumeScanned is calculated by multiplying the scan length in cm (from the bench sheet) by the Microscope Field Diameter in cm (found in the "Microscope Lenses" table of the PHYCLGY database) and then multiplying by 0.04, the depth of a Palmer-Maloney Cell in cm.

6.9.1.8. **Analysis Form ID, Number Counted** and **Corresp H_2O** Sample are not required or not applicable.

6.9.1.9. **Mag Changer** should be entered if applicable or if not applicable, = 1.

6.9.1.10. **Count Notes** is "Y" or "N" depending on whether there is a count note associated with the analysis.

6.9.1.11. **Validated, Validated By** and **Date Count Validated** will be entered, probably by the analyst, after verification of data entry.

6.9.1.12. **Phytoplankton Apparatus ID** is found in the "Phytoplankton Apparatus" table of the "PHYCLGY" database and refers to the code for the type of counting (Palmer-Maloney) or settling chamber (HydroBios, PhycoTech, etc.) used (and listed on the bench sheet).

6.9.2. Table "Non Diatom Count." There is one record for each taxon observed during the analysis. The following fields are entered for each record:

6.9.2.1. **Entry Order** is automatically incremented as data are entered.

6.9.2.2. **SampleID, SubSampleID, P-MFractionID, CountReplicateID** describe the sample and fraction used in the analysis.

6.9.2.3. **TaxonID** is the NADED Taxon Code for the taxon being entered.

6.9.2.4. **NumberCounted** field represents the number of natural units enumerated. The **NumberCells** is the total of number of cells for the specimens enumerated. The **NumberCells** is equal to the **NumberCounted** for diatoms and forms that are unicellular.

6.9.2.5. **RowNumber** is not entered for these analyses.

6.9.2.6. **TaxaNote** is "Y" or "N" depending if there is a taxa note for this particular taxon in this analysis.

6.9.3. The table "Biovolume Measurements" in the PHYCLGY database is updated by running the application "BioVol" (current version is 1.0.4) and entering data from the "Algal Biovolume Measurements" form.

6.10. **Calculation of phytoplankton abundances and biovolumes.** The calculation of phytoplankton abundance depends on the apparatus used during analysis. Biovolume values are determined by multiplying the abundance (cells/ml) by the average biovolume of each cell (μm^3). The average biovolume of each cell is determined by averaging all values for the taxon in the "Biovolume Measurements" table of the PHYCLGY database. If there are no records in the "Biovolume Measurements" table for the taxon, the program performing the calculation will use a predefined constant based on genus (for diatoms) or algae type (for non-diatoms). Equations for abundance calculations are given below. The calculations are performed at the time data are prepared for transmission to the NAWQA BioTDB. The "BioTDB export" application, written in MS Visual Basic, produces a table ("export_NAWQAResults") on the Phycology Section server (SQL; "Diatom"). This table, which contains the calculated biovolumes, can be accessed by other Phycology Section databases.

6.10.1. If the inverted microscope method was used in the analyses, phytoplankton abundance (cells/ml) is calculated as follows:

$$\text{cells/ml} = \frac{\text{count x field dcf x chamber area x subsample dcf}}{\text{microscope fld area x \# of microscope fields x chamber vol used}}$$

where:

count = "# cells" in the "Non Diatom Count" table; for diatoms, the number of cells is determined by dividing the "# cells" in the "Diatom Count" table by the total number of diatom cells enumerated and multiplying by the number of "<undifferentiated diatoms>" in the "Non Diatom Count" table.

field dcf = "DC Factor" in the "Sample Volumes/Areas" table.

chamber area = "Chamber Area" in the "Phytoplankton Apparatus" table (in mm^2).

subsample dcf = "D/C Factor" in the "Subsample Information" table.

microscope fld area = ["Lense Fld Dia" from the "Microscope Lenses" table (in μm)] divided by 2000, squared and multiplied by Pi (will be in mm^2); use the "Lense ID" in the "Non Diatom Count Information" table to get the correct microscope lense that was used.

number of microscope fields = "#Fields" in the "Non Diatom Count Information" table.

chamber volume used = "FractionVolume" from the "Palmer-Maloney Fractions" table.

6.10.2. If Palmer-Maloney counting cells were used in the analyses, phytoplankton abundance (cells/ml) is calculated as follows:

$$\text{cells / ml} = \frac{\text{count x field dcf x subsample dcf x P - M dcf}}{\text{volume scanned}}$$

where:

count, field dcf and subsample dcf are defined as above.

P-M dcf = "D/C Factor" in the Palmer-Maloney Fractions" table.

volume scanned = "VolumeScanned" in the "Non Diatom Count Information" table.

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

PHYTOPLANKTON COMMUNITY COMPOSITION Bench Sheet
(USGS NAWQA Program)

bench sheet #: **ANSP-GS-02-____-PP** project code: **GS708130**

study unit: **2001 Miami River Basins (MIAM)** subproj: **ANSP-GS-01-02-PP**
microscope: **L1015** lens: **Zeiss223165** PP apparatus: **4** count type: **38**
analyzed by:________________ __/__/**2002** worker id: **24** address id: **1**

	ANSP sample ID: **GSN** subsample-fraction: # fields:	**82**____ **PP1-**__ ____	**82**____ **PP1-**__ ____	**82**____ **PP1-**__ ____	**82**____ **PP1-**__ ____	**82**____ **PP1-**__ ____	**82**____ **PP1-**__ ____
blue-greens (Cyanophyta)							
dinoflagellates (Pyrrophyta)							
greens (Chlorophyta)							

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Figure 1. Phytoplankton community composition bench sheet (USGS NAWQA Program).

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bench sheet #: **ANSP-GS-02-_____-PP** project code: **GS708130**

ANSP sample ID: **GSN**		**82____**	**82____**	**82____**	**82____**	**82____**	**82____**
yellow-greens (Chrysophyta)							
other phytoplankton phyla							
diatoms (Bacillariophyta)							
249999	<undifferentiated live>						
249998	<dead or empty frustules>						
# **counting units (cells)**:		______	______	______	______	______	______

notes: ____

data entry by:_____ ___/___/**2002** confirmed by:_____ ___/___/**2002**

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Figure 1 (cont'd). Phytoplankton community composition bench sheet (USGS NAWQA Program).