*ACADEMY OF NATURAL SCIENCES OF DREXEL UNIVERSITY*
*PATRICK CENTER FOR ENVIRONMENTAL RESEARCH*

Procedure No. P-13-39 Revision 1(03/02)
Revision 2 (02/17)

Draft

# IDENTIFICATIONAND ENUMERATION OF DIATOMS ON MICROSCOPE SLIDES

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# Protocol P-13-39

# Identification and Enumeration of Diatoms on Microscope Slides

## 1. PURPOSE

1.1. This protocol describes Phycology Section procedures for analyzing diatom samples. It applies to the major kinds of samples analyzed, including those collected by local, state and federal agencies and the National Ecological Observatory Network (NEON) program. The previous version of this protocol (Revision 1, 03/02) describes procedures used in the Phycology Section from 2001 through about 2017, in particular for analysis of samples for the U.S.G.S. National Water-Quality Assessment program (NAWQA).

1.2. This protocol describes procedures for analyzing diatoms on microscope slides made from three types of algae samples; quantitative periphyton, qualitative periphyton, and phytoplankton. [These correspond, respectively, to the Richest Targeted Habitat (RTH) and Depositional Targeted Habitat (DTH), Qualitative Multihabitat (QMH), and phytoplankton samples (Porter et al., 1993; Moulton et al., 2002), collected as part of the U.S. Geological Survey's (USGS) National Water-Quality Assessment Program (NAWQA).]

1.2.1. The purpose of periphyton 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 can later be 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 $cm^2$ of sampling surface) and amount of algal biovolume ($\mu m^3$ per $cm^2$ of sampling surface) at a sampling site.

1.2.2. The purpose of the qualitative 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 the count of a periphyton sample, 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.

1.2.3. The purpose of phytoplankton sample analysis is similar to that of periphyton 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.

## 1. SCOPE

*2.1.* This protocol covers the identification and enumeration of diatom taxa mounted on microscope slides and procedures for recording data using the "Tabulator" program (Cotter 2002). As of 2017, all Phycology Section diatom analysts use the "Tabulator" program. 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 overview of the program and to help clarify how it is used in the process of analyzing samples. Some 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. It is applicable to periphyton and phytoplankton samples collected as part of the Neon 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.

## *2.* REFERENCES

*3.1.* Cotter, P. 2002. "Tabulator" Installation and User's Guide. Version 3.51. ANSP, PCER.

*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.* PCER, ANSP. 2002. Diatom Cleaning by Nitric Acid Digestion with a Microwave Apparatus. Protocol No. P-13-42.

*3.4.* PCER, ANSP. 2002. 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.

*3.5.* PCER, ANSP. 2002. Preparation of Diatom Slides Using Naphrax$_J$ Mounting Medium. Protocol No. P-13-49.

*3.6.* 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.

*3.7.* 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_home.html.

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

[add references for NEON periphyton and phytoplankton field sampling protocols; EPA NARS field sampling methods; Version 2 of NAWQA field protocols]

## 3. 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;

*4.1.3.* DIC (differential interference contrast) or bright field condenser;

*4.1.4.*

*4.1.5.* High intensity light source.

*4.1.6* Digital camera – (e.g., Leica DFC 3000G with Software LAS V4.6)

*4.2.* **Desktop computer (located at microscope).**

*4.2.1.* Software: "Tabulator" program by Patrick Cotter (MS Visual Basic);

*4.2.2.* Network connection to ANSP Phycology Section databases (ANSP staff only).

## 4. 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 important to clearly document the areas of a slide scanned during a count. After the preliminary slide examination, secure the slide in the mechanical stage and record the stage micrometer coordinates of the edge of the first transect (row or column) to be counted (the format should be the following: start x=nn.n, y=nn.n; end x=nn.n, y=nn.n). Transects are narrow, horizontal or vertical, rectangular areas (strips) of the slide with width equal to the field of view. Start transects far enough from the coverslip edge to avoid optical distortion and areas with uneven distribution of diatoms, and end them near the opposite coverslip edge, again avoiding marginal areas. Record the coordinates of the last field counted, and the beginning and end of all other transects. Record the coordinates in the Tabulator count note. In the count note of the first of a series (e.g., subproject) of samples, you may want to record your typical procedure for positioning a count transect (e.g., Start point in the center of the upper -as seen under the microscope- part of the slide, far enough from the slide edge to avoid area with uneven distribution of diatoms. Then vertical transects - columns- as needed).

*5.1.4.* When 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 transect should be recorded.

*5.1.7.* As the count commences, enter taxa observed using the discrete three-digit codes established during the taxa list building process or directly selecting taxa from the list in the right part of the screen by clicking on the taxon name. Enter codes with the numeric keypad on the computer keyboard or click on the taxon name the numbers of times needed. Record multiple examples of a single taxon 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 geographic location.

*5.1.7.1.* Quantitative periphyton analysis. Count 600 valves. The number of valves counted should be within 600 + 5%. To avoid exceeding the target value for > 30 valves (5%), in dense samples count only half or a quarter of the last field of view. Samples which are still very sparse after all possible expedients have been applied during diatom slide preparation (concentrating etc.) should be treated as an exception. For these samples apply the following. VERY-SPARSE SAMPLES COUNTS: Stop counting immediately if there are < 20 valves in a 12 mm transect. Otherwise count for 4 hours or count 6 transects ( average length = 12 mm; total length of all transects 72 mm), whichever comes first. The minimum target (total number of valves counted for these very-sparse samples counts) should be 100 valves. If, after counting for 4 hours the total number of valves counted is between 500 and 600, continue counting until the 600 valves target is reached. 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.* Qualitative multi-habitat (QMH) periphyton 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. Examine a minimum of 10 intervals (1000 individuals). 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 $9^{th}$ and $10^{th}$ intervals contain two or fewer new taxa, then stop the analysis at the end of the $10^{th}$ interval. Otherwise, continue the analysis until 2 consecutive intervals are found for which no more than 2 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 Diatom Prep Lab 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.

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.

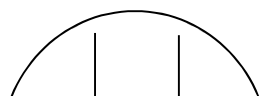
The number of measurements made for a taxon are shown in the field labeled "# of Measurements this session." If measurements must be corrected, click the "Datasheet" button at the bottom of the form and make changes in the appropriate record. After all measurements for a taxon have been entered, press 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 project. In general, take good quality digital micrographs of new, unknown, unusual and outstanding diatom specimens. This allows comparison with reference specimens and facilitates examination by specialists. For special purposes, specimens may be circled with a diamond scribe. 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



specimen location. Record extra information concerning documentation in the "Note" box, if necessary. You can click the "Datasheet" button to review records for existing circles.

| | | | |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 5 | 6 | 7 | 8 |
| 9 | 10 | 11 | 12 |

Cover Slip Sector

| | | |
|---|---|---|
| 1 | 2 | 3 |
| 4 | 5 | 6 |
| 7 | 8 | 9 |

Circle Sector

*5.3.3.* 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" B Taxon name and dimension 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" B 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" B 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 identification 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" is 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 FileName" recorded previously.

## 6. QUALITY ASSURANCE/QUALITY CONTROL

*6.1.* Sample and slide quality can affect the outcome of using these procedures. Minor deviations that do not affect the area scanned or number of specimens observed should be described in the Note portion (click the "Note" button) of the "Tabulator" program. Other deviations should be discussed with the Phycology Section Diatom Taxonomic Coordinator for inclusion in the project QA/QC notes.

*6.2.* Typically, 10% - 15% of the samples collected as part of a study will be analyzed for quality control. Two types of QA/QC analyses are commonly performed: 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 THCs are performed on diatom samples only while the RSCs are performed on both diatom and non-diatom samples. Descriptions of specific procedures are in QA/QC protocol documents.

## Diatom Slide Analysis Form - NAWQA

**For use with Protocol P-13-39 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
**Subproject ID:** ANSPGS0101PR
**Study Unit:** Acadian-Pontchartrain (ACAD 2001) USGS NAWQA

**Type of Sample/ Count:** RTH / DTH (600 Valves/300 Cells), QMH or Phytoplankton
**Analyst:**
**ANSP Account Number:** 708-2302

| Sample ID | Client Sample ID | Sample Type | Slide Replicate ID | ul dripped | Date Completed | Initials | Site Name |
|---|---|---|---|---|---|---|---|
| GSN71198 | ACAD0301ARE0007B | RTH | a b h l | 100 | ___/___/ 20__ | _____ | Bayou Lacassine nr Lake Arthur, LA |
| GSN71200 | ACAD0301AQE0007B | QMH-micr | a b h l | 150 | ___/___/ 20__ | _____ | Bayou Lacassine nr Lake Arthur, LA |
| GSN71205 | ACAD0301ADE0021B | DTH | a b h l | 100 | ___/___/ 20__ | _____ | MERMENTAU RIVER @ MERMENTAU, LA |
| GSN71208 | ACAD0301ADE0022R | DTH | a b h l | 100 | ___/___/ 20__ | _____ | MERMENTAU RIVER @ MERMENTAU, LA |
| GSN71211 | ACAD0301ARE0021B | RTH | a b h l | 100 | ___/___/ 20__ | _____ | MERMENTAU RIVER @ MERMENTAU, LA |
| GSN71213 | ACAD0301ARE0022R | RTH | a b h l | 25 | ___/___/ 20__ | _____ | MERMENTAU RIVER @ MERMENTAU, LA |
| GSN71215 | ACAD0301AQE0021B | QMH-micr | a b h l | 100 | ___/___/ 20__ | _____ | MERMENTAU RIVER @ MERMENTAU, LA |
| GSN71217 | ACAD0401ADE0023B | DTH | a b h l | 80 | ___/___/ 20__ | _____ | BAYOU DES CANNES NR EUNICE, LA |
| GSN71219 | ACAD0401ARE0023B | RTH | a b h l | 400 | ___/___/ 20__ | _____ | BAYOU DES CANNES NR EUNICE, LA |
| GSN71221 | ACAD0401AQE0023B | QMH-micr | a b h l | 400 | ___/___/ 20__ | _____ | BAYOU DES CANNES NR EUNICE, LA |
| GSN71223 | ACAD0401ADE0025B | DTH | a b h l | 100 | ___/___/ 20__ | _____ | WHISKEY CHITTO CK NR OBERLIN, LA |
| GSN71225 | ACAD0401ARE0025B | RTH | a b h l | 100 | ___/___/ 20__ | _____ | WHISKEY CHITTO CK NR OBERLIN, LA |
| GSN71227 | ACAD0401AQE0025B | QMH-micr | a b h l | 100 | ___/___/ 20__ | _____ | WHISKEY CHITTO CK NR OBERLIN, LA |
| GSN71229 | ACAD0401ADE0027B | DTH | a b h l | 50 | ___/___/ 20__ | _____ | DAWSON CREEK AT BLUEBONNET BOULEVARD |
| GSN71231 | ACAD0401ARE0027B | RTH | a b h l | 100 | ___/___/ 20__ | _____ | DAWSON CREEK AT BLUEBONNET BOULEVARD |
| GSN71233 | ACAD0401AQE0027B | QMH-micr | a b h l | 50 | ___/___/ 20__ | _____ | DAWSON CREEK AT BLUEBONNET BOULEVARD |

**PREPARED SLIDES TRANSMITTED BY:** _____ **Date:**___/___/___

**PREPARED SLIDES RECEIVED BY:** _____ **Date:**___/___/__

**DATA AND SLIDES TRANSMITTED BY:** _____ **Date:**___/___/____

**DATA AND SLIDES RECEIVED BY:** _____ **Date:**___/___/__

*March 29, 2002*

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**Figure 1.** Diatom Slide Analysis form - NAWQA.