Phytoplankton Diatom Identification and Species Proportional Counts

(Modified from Standard Methods 10300C.3)

1. SCOPE AND APPLICATION

1.1 This method details the counting rules and procedures for identifying diatoms in phytoplankton samples.

1.2 This method is used for quantitative phytoplankton samples collected by any method.

1.3 This method is to be used for all samples with Laboratory Information Management System (LIMS) test codes beginning with “DTY”.

2. SUMMARY OF METHOD

2.1 The number of diatoms valves to be identified on the prepared slide is determined based on the type of sample.

2.2 If the sample is a non-Everglades sample, 500 valves are identified except under special circumstances where 500 valves are not available on two prepared slides. In that case, identification is terminated, and a comment is recorded on the bench sheet.

2.3 For Everglades samples, the number of valves identified depends on the number of live frustules counted in the wet ID process. This is a modification of the Standard Method 10300C.3 valve count.

2.4 Location and identification to the lowest practical level of diatoms on the slide is accomplished by starting at the top left edge of the slide and proceeding in transects across the slide from left to right. When the right edge of the slide is reached, return to the left edge and move the objective down to start a new transect. This ensures that as many valves as possible are encountered and that the same valves are not counted and identified twice.

3. APPARATUS AND EQUIPMENT

3.1 Compound microscope (Leica DM LB with Differential Interference Contrast [DIC])
3.2 Immersion oil
3.3 Laboratory counter
3.4 Black ink pen
3.5 Phytoplankton Diatom Bench Sheet

4. SAMPLE COLLECTION AND PRESERVATION

4.1 Samples are collected, preserved, and delivered to the lab according to FS 7000.

5. SAMPLE PREPARATION PROCEDURE

5.1 Phytoplankton samples are checked into the lab and processed per SOP AB-11.

5.2 Samples are prepped per SOP AB-02.1.

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6. SAMPLE ANALYSIS PROCEDURE

6.1 Slides of cleared diatom frustules are examined under oil immersion at a magnification of 1000x.

6.2 Record the following information on the Phytoplankton Diatom Bench Sheet: analyst and analysis date. For Everglades samples, record in the comments section of the bench sheet the number of diatoms counted in the original phytoplankton sample (wet ID).

Note: The number of diatoms counted in the wet ID of the phytoplankton sample can be found on the phytoplankton non-diatom bench sheet found in the folder.

6.3 Determine the target number of valves to count for the type of sample being identified.

- For all **non-Everglades** phytoplankton sample, the number of valves to identify will be **500**.
- For the **Everglades** samples, determine the number of valves to identify based on the number of diatom frustules counted in the wet ID analysis. Record the target in the comments section on the bench sheet.

  a. If the number of frustules counted in the wet ID is <50, double the wet count and identify this number of valves on the prepared slide.

  b. If the number of valves counted in the wet ID is >=50, identify 500 valves.

Note: Diatoms identified in the wet ID are only those containing a chloroplast, and therefore are assumed to be whole diatoms with both valves or sides present. Whole diatoms are referred to as “frustules”. Identifications performed on the diatom slides are considered identifications of diatom “valves” since many diatoms will break apart during the clearing process. By focusing up and down, one can see if both valves of a diatom are present (a count of 2 valves) versus one valve of a frustule that has broken apart (a count of 1 valve).

6.4 Some diatom frustules do not survive the clearing process. These are to be counted and recorded during wet ID on the wet ID bench sheet. If such diatoms are found during diatom analysis, they should only be recorded on the diatom bench sheet if they were not recorded on the wet ID bench sheet.

Note: Some examples of diatoms that may not survive the clearing process (SOP AB-02.1) are *Rhizosolenia, Odontella, Skeletonema, Leptocylindrus, Attheya,* and *Chaetocerus.* Additional taxa that have been identified in wet ID using phase contrast but not in diatom analysis and therefore may need to be added to this list include *Cylindrotheca closterium, Bacteriastrum delicatulum, Cerataulina* sp., *Cerataulina bicornis*, and *Dactyliosolen blavyanus*. This has been determined in previously identified samples. These diatoms tend to be very lightly silicified and usually too fragile for the boiling process. However, some species or species under certain environmental conditions may survive boiling and show up on the slide. If these particular species are rare in the sample, they may not have been encountered in the wet ID.

6.5 Beginning on the top, left side of the slide, scan a lateral strip, identifying each valve to the lowest practical taxonomic level. Record each taxon, along with the number of valves counted, on the appropriate Phytoplankton Diatom Bench Sheet. Enumeration (recording the numbers of valves of each taxon) occurs concurrently using a laboratory counter.

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Note: Always proceed from left to right to avoid counting any areas twice. When you reach the right side of the coverslip, return the objective all the way to the left side of the coverslip, move down slightly, and then resume scanning for diatoms moving again to the right.

The compound microscopes project images that are the inverse of actual movement across the slide. Personal preference can be exercised when deciding to move based on actual slide movement or movement as it is perceived through the eyepieces.

Note: If a diatom is encountered intact (i.e. both valves are present), it should be counted as two valves. If only one valve is present, it should be counted as one.

6.6 Occasionally, the targeted number of diatom valve counts cannot be obtained with a single diatom slide. In these cases, a second slide should be prepared and counted in an attempt to reach the targeted number. Make a note of this situation on the bench sheet. If the target cannot be obtained using two slides, stop, and notify the Taxonomy Supervisor.

Note: In isolated cases, diatoms that were alive at the time of sampling and counted in the wet ID do not survive the clearing process well. The reasons for this phenomenon are unknown at this time. It is suspected that it may have to do with weakening of the diatom frustules from prolonged storage in formalin and/or properties of the water body from which the algal sample is collected. In this case, although 500 valves should be ID'd, it is impossible to achieve this count without preparing numerous slides and spending excessive time analyzing one sample.

6.7 Total the number of diatom taxa; do not include diatom taxa identified in the wet ID in this diatom taxa count. At the bottom of the bench sheet, record the total taxa and the total number of slides used. At the top of the bench sheet, record the analysis date, analysis time, and the total diatom valve count.

6.8 In the appropriate logbook(s), record the necessary information for the completed sample.

6.9 Entry, verification, and authorization of taxonomic data in Statewide Biological Database (SBIO) and LIMS is performed per SOPs BG-03 and BG-01 by individuals designated by the Taxonomy Supervisor.

7. DATA ARCHIVAL

7.1 Samples and slides are kept for 5 years, after which time they may be discarded using appropriate safety procedures. Bench sheets are filed by project and kept indefinitely.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all logbook and bench sheet entries.

8.2 Phytoplankton Diatom Bench Sheet printed from LIMS

8.3 DEP Laboratory Information Management System (LIMS)

8.4 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error,initial and date the correction. If the correction requires an explanation, write a comment and reference the error.

8.5 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create a NCR, open LIMS and go to

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Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

8.6 Refer to the Laboratory Quality Manual.

9. SAFETY

9.1 Refer to the Laboratory Health and Safety Plan.

10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 Refer to the Laboratory Health and Safety Plan.

11. REFERENCES

11.1 DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/lab_sops.htm

11.2 DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

11.3 SOP FS-7000, *General Biological Community Sampling*

11.4 SOP AB-02.1, *Diatom Preparation for Periphyton and Phytoplankton*

11.5 SOP AB-11, *Phytoplankton Sample Receipt and Sample Handling for Taxonomic Analysis*

11.6 SOP BG-01, *Data-Entry Verification*

11.7 SOP BG-03, *Taxonomic Results and Data Entry*

11.8 LB-002, *Non-Conformance Reporting System*

Appendix of Changes

09/27/2010 Reformatted the layout following SOP LB-001. Removed instructions for LIMS Custody Utility. Added Data Archival.

09/28/2015 Included the specific modification from SM 10300C.3 in the Summary of Method section. Moved much of the information previously in Data Archival section into SOP BG-03. Updated Lab Health and Safety Plan link. Formatted for 508 compliance.

09/29/2016 Updated Laboratory Quality Manual link. Added language to Section 5 Sample Preparation Procedures that referenced SOP AB-11, which was previously not cited in the text. Edited the citation for SOP FS-7000 to remove the SOP title to make all citations consistent.

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