Analyses of Algal Bloom and Algal Identification Samples

(Modified from *Standard Methods 10200F* and *10900C*)

1. SCOPE AND APPLICATIONZ

1.1 This method details the rules and procedures for identifying the dominant taxon (or co-dominant taxa) for non-diatom algae in phytoplankton and periphyton bloom samples.

1.2 This method is used for qualitative phytoplankton samples collected by the methods established in SOP FS 7000, *General Biological Community Sampling* (FS 7110, *Phytoplankton Bloom Sampling* and FS 7240, *Algal Mat Sampling for Taxonomic Identification or Toxin Analysis*).

1.3 This method is to be used for samples with Laboratory Information Management System (LIMS) test codes “BLOOM-ID” and “ALGAL-ID”.

1.4 This test was developed for identification only; no attempt should be made to determine whether a sample looks like it represents an algal bloom. If the customer determines more information is needed, a full wet ID (PKD-QN-C/DTY-QN-C analyses) should be scheduled.

2. SUMMARY OF METHOD

2.1 The BLOOM-ID or ALGAL-ID sample is checked out from Receiving and taken to the Algal Lab.

2.2 Phytoplankton samples are inverted gently to mix well. Mats and filamentous algae do not require mixing.

2.3 The phytoplankton method uses a modified version of SM 10200F.2. An Utermöhl counting chamber is used for algal identification and determination of taxa abundance, not for quantifying taxa. Phytoplankton identifications in the wet material are performed at 400X to 450X magnifications. Dilutions are not required. Periphyton identifications are performed at 5X-40X magnifications.

2.4 The dominant taxon (or co-dominant taxa) is identified and reported.

3. APPARATUS AND EQUIPMENT

3.1 Inverted microscope (calibrated and maintained per SOP AB-01)
3.2 Disposable transfer pipettes
3.3 Glass slides
3.4 Utermöhl counting chamber
3.5 Cover slips, 24 x 60 mm
3.6 Petri dishes, 100 x 15 mm
3.7 Forceps
3.8 Filter paper to fit Petri dish
3.9 Laboratory counter (not required)
3.10 Labeling tape and marker
3.11 Safety equipment (see section 10)

4. REAGENTS AND CHEMICALS

4.1 Lugol's Solution (prepared per SOP AB-07)

5. SAMPLE COLLECTION AND PRESERVATION

5.1 Samples are collected, preserved, and delivered to the lab according to SOP FS 7000 (Section 7110, *Periphyton Bloom Sampling* and Section 7240, *Algal Mat Sampling for Taxonomic Identification or Toxin Analysis*).

6. SAMPLE RECEIPT AND HANDLING

6.1 BLOOM-ID and ALGAL-ID samples may arrive in a variety of container types. They can be collected when a bloom is observed during routine sampling events and for which sampling was not necessarily planned (i.e. scheduled in LIMS). They may also be collected when a quick response is needed due to a reported event and there is not time to schedule and receive a kit from the DEP Lab in Tallahassee.

6.2 Custody – Check Out

6.2.1 Periphyton: Follow SOP AB-10.1, Steps 6.1.1-6.1.3, for sample check-out procedures.

Phytoplankton: Follow SOP AB-11, Steps 6.1.1-6.1.3, for sample check-out procedures.

6.3 Custody – Check In

6.3.1 Follow SOP AB-10.1, Step 6.1.2 (periphyton) or SOP AB-11, Step 6.1.2 (phytoplankton), replacing **CheckOut button** with **CheckIn button**.

6.4 Preservation and Hold Times

6.4.1 Do not chemically preserve BLOOM-ID or ALGAL-ID samples.

6.4.1.1 If the sample needs to be kept after analysis, the sample can be preserved with buffered formalin (periphyton, prepared per SOP IZ-10) or Lugol's (phytoplankton) after the sample has been viewed by an analyst to determine the dominant species.

6.4.2 Per DEP SOP FS 7000, bloom samples should be kept in the dark and on ice. They have up to 36 hours from time of collection to be analyzed; however, SM 10200 allows for samples to be stored in a dark refrigerator if they are not going to be shipped immediately after collection or analyzed immediately upon receipt.

6.4.2.1 If the algae have not been kept cool or in the dark prior to receipt and they are degraded and unidentifiable, the customer will be contacted and informed that the sample cannot be analyzed. It will be reported as "No Result" with an "O" qualifier. A non-conformance report (NCR) will be created in LIMS.

6.4.2.1.1 When the result is entered into LIMS, include the comment "See non-conformance report #__." (Fill in the blank with the appropriate number.)

6.4.2.2 If samples arrive beyond 36 hours from time of collection due to shipping delays, or if the samples are out of temperature range for short-hold samples, Receiving will create an NCR.

6.4.2.2.1 If the algae are not degraded and the sample can be analyzed, find the Receiving NCR and add a comment to the resolution: "The algae were not degraded and were able to be analyzed. The result will not be qualified."

6.4.2.2.2 If the algae are degraded and unidentifiable, find the Receiving NCR and add a comment to the resolution: "The algae were degraded and were unable to be identified. The sample will be reported as "No Result" with an "O" qualifier."

6.4.2.2.2.1 When the result is entered into LIMS Results Editor, include the comment "See non-conformance report #__." (Fill in the blank with the appropriate number.)

7. SAMPLE ANALYSIS PROCEDURE

7.1 Periphyton and algal mat samples:

7.1.1 Using forceps or gloved hands, place periphyton in a petri dish.

7.1.2 Take a small portion of the periphyton from the dish and place it on a slide. Place a coverslip on the slide.

7.1.3 Place the slide on the stage of the inverted microscope.

7.1.4 Use appropriate objectives to identify the periphyton to the lowest possible taxonomic level.

7.1.5 Repeat step 7.1.2 and analyze another portion of the sample. Repeat 2 -3 times to ensure the dominant taxon or co-dominant taxa have been represented. Other taxa may also be identified.

7.1.5.1 For other taxa present, scan the sample and identify the taxa to the lowest possible taxonomic level. If there is an abundance of taxa, record the most common taxa.

7.1.6 Record the dominant taxon or co-dominant taxa and the other taxa on the BLOOM-ID/ALGAL-ID benchsheet. If the sample was not periphyton, note that on the benchsheet. Complete the benchsheet with sample analysis information, including sample/sample container observations, microscope ID, magnification (power) used for identification, grid length, other taxa present, and total wet taxa.

7.1.6.1 Note any distinctive or unusual characteristics about the sample, such as color or smell.

7.1.6.2 Although grid length is not needed for analysis of qualitative samples, it is good practice to record it on the benchsheet.

7.2 Phytoplankton samples:

7.2.1 Prepare Petri dish/es. Place two pieces of filter paper into a Petri dish and moisten with DI water. Place an Utermöhl counting chamber on the moistened filter paper.

Note: The Petri dish creates a moist environment while the sample is settling, thus avoiding the formation of an air bubble due to evaporation. Be sure that the filter paper is smooth and that the Petri dish is placed on a level surface during settling. Otherwise,

the algal cells will tend to settle out on one side of the Utermöhl counting chamber and will not be evenly distributed.

7.2.2 Mix the contents of the sample by gently inverting the centrifuge tube 3 or 4 times. If the gentle inverting of the tube does not dislodge all settled material from the bottom of the tube, invert a little more vigorously. Be careful of shaking the sample too vigorously, however, as this tends to break up some blue-green colonies.

7.2.3 Using a transfer pipette, fill the cell of the Utermöhl counting chamber with the well-mixed sample so that the liquid is barely higher than the sides of the Utermöhl counting chamber. Using a designated transfer pipette, add 1 drop of Lugol's solution to the counting chamber. Starting at an angle, carefully lay a cover slip over the cell opening to ensure that no air bubbles are introduced.

7.2.4 Cover and label the Petri dish with LIMS Sample ID and time prepped. Let the sample settle just long enough to allow proper identification, typically 1 hour.

Note: In order to give quick turnaround on results, the sample may be identified before the sample has settled for 1 hour if the analyst determines accurate identification of the dominant taxon or co-dominant taxa is possible.

If the dominant taxon or co-dominant taxa have been identified after the sample has settled for less than 1 hour, the sample may need to settle for more time in order to identify other taxa. Use professional judgement.

7.2.4 Remove the counting chamber from the Petri dish and carefully wipe away any excess moisture, particularly on the underside. Place counting chamber on the stage of the inverted microscope.

Note: Be careful not to tip the counting chamber or bump it as this will cause the algal cells to dislodge from the bottom and float, making accurate identification difficult.

7.2.5 Position the stage so that the left edge of the counting chamber is in view. Move a short distance to the right and observe all algae within the field of view. Always move from left to right across the cell during the progression to a new field. This eliminates the possibility of accidentally changing directions and viewing fields more than once.

7.2.6 At 400X magnification, identify non-diatom algae to the lowest possible taxonomic level. Only algae that appear to have been alive at the time of collection should be counted. Algal cells/colonies/filaments without chloroplasts should NOT be identified.

7.2.7 For BLOOM-ID and ALGAL-ID samples, three to five fields of view are counted. Three fields of view for typical to high algal density samples, five for low algal density samples. The number of algal units are recorded on the benchsheet for each of the most prevalent species for each field of view. An average number of units is then calculated for each species identified in the fields. There can only be two co-dominant taxa. If three or more taxa occur at nearly the same quantity, then "no clear dominance" is recorded on the benchsheet. Comparing averages and taxon abundance, professional judgement is used to determine which species are dominant, co-dominant, or if there is no clear dominance.

7.2.7.1 As a general rule, if one taxon has an average greater than five, then that is the dominant taxon. If two taxa have an average greater than five, then they are co-dominant. If three or more taxa have an average greater than five, then the two

highest average taxa are co-dominant. Based on the overall abundance of each taxon, professional judgement is used to determine the dominant taxon or co-dominant taxa.

7.2.7.2 The sample should be well homogenized before analysis; however, some large colonies of taxa, such as *Microcystis* sp., do not homogenize easily. Also, samples with low taxa density or diversity, even if they have been well mixed, may not be fully represented in the chamber slide. Professional judgement based on abundance is used to determine dominance.

7.2.7.3 Some algae colonies are visible in the sample to the naked eye as floating algal specks. If large specks (easily visible to the naked eye) and/or a high density of specks are observed in the sample, and there are no other taxa present in high levels in the Utermöhl chamber, then the observed taxon is recorded as dominant. If large specks and/or a high density of specks are observed and another taxon is dominant in the Utermöhl chamber, then the taxa are recorded as co-dominant. In any case, specks should be removed and mounted on a slide to determine identity.

7.2.7.4 If specks are fine (less than ~2.5 µm), then they will likely be captured with the transfer pipette while the Utermöhl chamber is being prepped and settled, and will be analyzed with the rest of the phytoplankton. If specks are very low density (guideline: less than 3 specks in the sample container), then a speck should be removed and mounted on a slide to determine identity.

7.2.7.5 Taxa that are observed but are not dominant are listed as “other taxa present.” If there is an abundance of taxa, record the most common taxa.

7.2.8 Record the dominant taxon or co-dominant taxa and the other taxa on the BLOOM-ID/ALGAL-ID benchsheet. If there is no dominant taxon or if the sample is not algae, note that on the benchsheet. Complete the benchsheet with sample analysis information, including sample/sample container observations, settling time, microscope ID, magnification (power) used for identification, grid length, cell depth, other taxa present, and total wet taxa.

7.2.8.1 Note any scum, specks, discoloration, smell, or any other distinctive or unusual characteristics about the sample.

7.2.8.2 Although the cell depth and grid length are not needed for analysis of qualitative samples, it is good practice to record it on the benchsheet.

7.2.8.3 Note and count diatoms observed in the fields of view, but do not include Bacillariophyta as an “other taxon present.”

7.2.8.4 Occasionally, a BLOOM-ID sample will require a dilution. Dilution information should be calculated and recorded. The information gives the analyst an overall idea of the density of the algae in the sample. Refer to SOP AB-05 for dilution calculation formulas.

Samples not requiring a dilution have a dilution factor of 1 (1x). It is recorded this way because of the way it is used in the density calculation cited in Standard Methods. This is also the density calculation used in the Statewide Biological Database.

7.2.9 Diatom identification is not normally performed for BLOOM-ID and ALGAL-ID samples. If diatoms are highly dominant and, after viewing the sample, it is suspected that the bloom taxon is a diatom, then the dominant diatom(s) are analyzed and identified after the non-diatom identification is completed.

7.3 Entry, verification, and authorization of taxonomic data in SBIO and LIMS is performed per SOPs BG-01 and BG-03.

7.4 The BLOOM-ID sample results are posted to the DEP Florida Algal Bloom website.

8. QUALITY CONTROL

8.1 QC of Taxonomic Identification is performed per SOP AB-14.

8.2 Refer to the Laboratory Quality Manual for all QC procedures and documentation.

9. DOCUMENTATION AND DATA ARCHIVAL

9.1 Use a pen with black ink for all logbook and benchsheet entries.

9.2 DEP Laboratory Information Management System (LIMS) and Statewide Biological Database (SBIO).

9.3 FDEP Algal Taxonomy Bloom-ID/Algal-ID benchsheet printed from LIMS.

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

9.5 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create an NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

10. SAFETY

10.1 Wear lab coat, latex or vinyl gloves, and eye protection as appropriate.

10.2 Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of chemicals. Work in a well-ventilated area.

10.3 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

10.4 Refer to the Laboratory Health and Safety Plan.

11. POLLUTION PREVENTION AND WASTE MANAGEMENT

11.1 Refer to the Laboratory Health and Safety Plan.

12. REFERENCES

12.1 DEP Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

12.2 DEP Laboratory Health and Safety Plan, https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx

12.3 *Standard Methods* 10200: *Plankton*; 10200F: *Phytoplankton Counting Techniques* (2017)

12.4 *Standard Methods* 10900: *Identification of Aquatic Organisms*; 10900C: *Key for Identification of Common Freshwater Algae (Plates 1A, 1B, 4A, 4B and 28-40)* (2017)

12.5 SOP AB-01, *Compound and Inverted Microscope Calibration*

12.6 SOP AB-07, *Preparation of Lugol's Solution*

12.7 SOP AB-10.1, *Periphyton Sample Receipt & Sample Handling for Taxonomic Analysis*

12.8 SOP AB-11, *Phytoplankton Sample Receipt & Sample Handling for Taxonomic Analysis*

12.9 SOP AB-14, *Algal Identification Quality Control (QC) Procedures: Initial Demonstration of Proficiency, Ongoing/Individual QC, Group QC, and Annual QC*

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

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

12.12 SOP LB-002, *Non-conformance Reporting System*

12.13 SOP IZ-10, *Preparation and Proper Disposal of Buffered Formalin*

12.14 SOP FS 7000, *General Biological Community Sampling* (FS 7110, *Phytoplankton Bloom Sampling* and FS 7240, *Algal Mat Sampling for Taxonomic Identification or Toxin Analysis*).

Appendix of Changes

12/16/2019 Removed BLOOM-ID/ALGAL-ID references from other algal SOPs upon the creation of this SOP.

09/03/2020 Edited Section 6.4 to include NCR comments.

08/31/2022 No changes.

08/24/2023 Removed sections 6.2.3, 7.3, and 9.3. Algal Logbook Labels are no longer printed and the Algal Logbook has been discontinued.

08/06/2024 Removed references to IZ-38 and changed to reference IZ-10 for preparation and use of formalin. Edited reference in Section 12.9 to match the current titles of the SOP. Removed instructions to print submittal sheets and instructions to create and send SBIO reports.

08/06/2025 Edited Section 7.2.7 to better clarify and address when to and when not to pull specks, as well as when to consider specks to be a dominant taxon in the sample. Also in 7.2.7, using fields of view was previously an option. Now, it is the method.