STANDARD OPERATING PROCEDURE FOR MACROALGAE IDENTIFICATION AND DATA SUBMISSION-ABBREVIATED

ALG 10006A

Method Reference: This method is based on Stancheva et al. 2015

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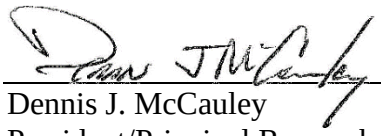
Great Lakes Environmental Center, Inc.
(GLEC)

3/5/2025

Julianne Heinlein — Date
Technical Approver



3/5/2025

Dennis J. McCauley — Date
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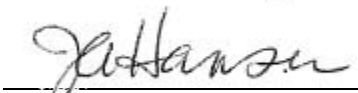

3/5/2025

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I. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the processing and taxonomic identification of macroalgae samples and draws heavily from Stancheva et al. 2015. Data submission for the National Ecological Observatory Network (NEON) macroalgae samples is also outlined.

1.2 This SOP may be modified by mutual consent of Great Lakes Environmental Center (GLEC) and the client to achieve the objectives of a given study plan.

II. SUMMARY OF METHOD

2.1 Macroalgae are large macroscopic filamentous, colonial, tuft-forming, crustose, tissue-like or coenocytic eukaryotic algae and cyanobacteria that have forms recognizable with the naked eye (from Stancheva et al. 2015). The purpose of this semi-qualitative analysis of macroalgae samples is to identify as many taxa present in the sample as possible and to estimate the relative percentage of each. All macroalgal taxa are identified to the lowest possible taxonomic level. Some species-level identification may require observation of different life stages to determine vegetative features, reproductive mode, and characteristics of completely developed reproductive structures of each species which may or may not be present in the sample. Identification will require observation under a compound and/or dissecting microscope.

2.2 The procedures in this SOP draw heavily from “Standard operating procedures for laboratory processing, identification, and enumeration of stream algae SWAMP BioAssessment Procedures 2015,” Stancheva et al. 2015.

III. INTERFERENCES AND CAUTIONS

3.1 Macroalgae may be damaged during manipulation so care must be taken when moving the specimens from one vessel to another.

3.2 Keep samples moist during analysis to avoid dehydration.

IV. EQUIPMENT AND SUPPLIES

6.1. Carboy for DI

6.2. 250 mL wash bottle with fine tip

6.3. Permanent markers and pens

6.4. Long-handled forceps and fine-tip, jeweler's forceps

6.5. Safety razor blade

6.6. Nitrile gloves

6.7. Laboratory trays

6.8. Petri dish

6.9. Microscope slides

6.10. Microscope coverslips

6.11. Compound and dissecting microscope with digital camera

V. REAGENTS AND STANDARDS

5.1 Reagent Water – DI Water

5.2 Reagents – Biological preservative - Glutaraldehyde

VI. SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1 Samples are collected by the client(s).

6.2 Sample Receipt

6.2.1 Macroalgae samples should have been received and recorded by a laboratory technician according to SOP ALG 10001A. If not, please refer to ALG10001A Section VII for sample check-in procedures.

6.3 Sample Preservation and Storage

6.3.1 Preserved samples should be kept in a sample refrigerator, at 4ºC, until processed. Long term storage of preserved materials may be at room temperature in a dark space.

6.4 Clean glassware, falcon tubes, petri dishes, and forceps in the following manner. Rinse with hot tap water and soak in Liquinox © 3% solution for a minimum of 4 hours. Rinse with tap water followed by triple rinse with DI water. Forceps must be rinsed and dried between each sample.

6.5 Observed loss of material at any stage of this procedure compromises quantitative samples. Samples which experience such loss should be discarded immediately if sufficient material has been retained to allow for a fresh sample. If the compromised sample is the only material available from the original sample, the procedure should be completed, and the nature of spillage or loss should be documented clearly.

VII. PROCEDURE

7.1 Macroalgae Processing

7.1.1 Check that each sample is intact and smell the sample to determine if any decay is occurring. If decay is detected, add additional appropriate preservative to all original samples and note this addition on the data sheets and on the data ingest table. NEON samples are preserved in glutaraldehyde.

7.1.2 Using the long-handle forceps, carefully collect macroalgal material from the sample tube and transfer to a glass dish for observation under the dissecting microscope. Search the sample tube for visible clumps of algae as well as other solid particles such as mosses and vascular plant tissue. Gently swirl the material clasped in the forceps in the sample liquid, prior to transfer, to remove extraneous sediment and to isolate different taxa. Do this until all macroalgae and solid particles are removed to the glass dish.

7.1.3 If no macroalgae or other solid material is visible to the naked eye, inspect the sample tube under the dissecting microscope to verify absence of macroalgae.

7.2 Macroalgae Identification and Relative Abundance Estimation

7.2.1 Under the dissecting microscope, examine the sample to determine the number of potential macroalgal species present. Search through all material for macroalgal features that are key to genus and species-level identification.

From Stancheva (2015): “These may include:

7.2.1.1 Colonial shape, size, and color in cyanobacteria (such as Nostoc, Dichothrix, Rivularia).

7.2.1.2 Different life stages, heterocyst position, and akinete development in cyanobacteria (such as Anabaena, Cylindrospermum, Gloeotrichia).

7.2.1.3 Male and female specimens with developed reproductive structures in red and green algae (such as Batrachospermum, Sirodotia, Oedogonium).

7.2.1.4 Different life stages and completely matured reproductive structures in zygnematalean algae and tribophytes (such as Spirogyra, Zygnema, Mougeotia, Vaucheria).

7.2.2 Set aside a representative sample of each potential species for slide preparation and further observation under a compound microscope. If small rocks are collected, examine their surface for attached algae. If algae are present, carefully scrape the specimen off using the forceps or a razor blade and transfer the material to a microscope slide.

7.2.3 Prepare microscope slides for each potential species. These slides should include diagnostic features when available. For this reason, you may require more than one slide per potential species.

7.2.4 Examine prepared slides under a compound microscope and identify to species-level when possible. As mentioned previously, some specimens may not contain all the required features for species-level identification. If species-level identification is not possible for a given genus, but species differences are apparent, track each probable species separately using the genus and a project-wide species number (e.g., Spirogyra sp. 1).

7.2.5 Large colonial diatoms are included in this analysis. If present, note the genus and separate this material from the other macroalgae.

7.2.6 Take enough digital images of each macroalgae taxon to aid with taxonomic consistency and harmonization. These images should include the features required for species-level identification.

7.2.7 Following the identification of all macroalgae taxa present, estimate each species' proportion of the total macroalgae biomass, their relative abundance. This should include large colonial diatoms if present. Use the scale below to assign a subjective rating, 1-6, to each taxon.

Table 1. Relative Abundance Rating

Rating	Relative Abundance
1	<10%
2	10-29%
3	30-49%
4	50-74%
5	75-99%
6	100%

7.3 Identified NEON macroalgae samples will be returned to the original, 60mL NEON sample containers, labeled with the sample ID and barcode they had upon arrival. Macroalgae samples will be preserved in 2% glutaraldehyde on arrival and may require additional preservative depending on condition. Change and add sample preservative as necessary for long-term storage. Storage should be in a dry, clean, well-ventilated area at room temperatures between 15° to 25°C or up to 30°C, depending on climatic conditions. After processing, all samples will be shipped to the NEON Biorepository at Arizona State University for archiving.

VIII. DATA ANALYSIS AND CALCULATIONS

8.1 Record the species name, taxonomic reference, relative abundance (estimated % of total macroalgae biomass) and any other required information for each taxon.

8.2 Data Recording and Submission to Client

8.2.1 NEON macroalgae data are to be recorded in the format presented in "Attachment 2c_algTax_Lab Data File". Field definitions are included in "Attachment 2b_algTaxonomy_fieldDescriptions."

8.2.1.1 Taxa reported must match the NEON taxon table located on the NEON Data Portal. Notify Battelle if additional taxa need to be added to the taxon table using "Attachment 2e_NEON_taxon_request_template" in advance of data return, in sufficient time for additions to be made during periodic (approximately monthly) updates to the data system.

8.2.1.2 The primary identification reference used to make the taxonomic determination must be entered as 'Identification References.'

8.2.1.3 Upload completed datasheets to the NEON Data Portal using the instructions provided in "Attachment 2a_uploading-files-to-NEON_AlgalTaxID".

IX. EQUIPMENT MAINTENANCE

Glassware, forceps, and falcon tubes are cleaned after each use by soaking in Liquinox © 3% solution for a minimum of 4 hours and rinsing with DI water.

X. QUALITY ASSURANCE

The laboratory manager will generate an Excel data file and/or summary report based on the client's instructions.

XI. PROTOCOL REFERENCES

11.1 GLEC SOP ALG 10001A. Algae Sample Receipt, Subsampling, and Diatom Sample Cleaning.

11.2 Stancheva, R., Busse, L., Kociolek, J.P. & Sheath, R. 2015. Standard operating procedures for laboratory processing, identification, and enumeration of stream algae SWAMP BioAssessment Procedures 2015: 1-100.

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