STANDARD OPERATING PROCEDURE FOR ALGAE SAMPLE RECEIPT, SUBSAMPLING, AND DIATOM SAMPLE CLEANING, ABBREVIATED

ALG 10001A

Method Reference: See References Section.

October 11, 2024

Great Lakes Environmental Center, Inc.
(GLEC)

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I. SCOPE AND APPLICATION

1.1 This Standard Operating Procedure (SOP) describes the sample receipt, subsampling, and archiving of algae samples for both soft algae identification and diatom identification. It also describes the microwave digestion of the diatom subsample using the ETHOS UP microwave digester to clean organic matter from the diatom cells.

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 Upon receipt of samples, their condition is assessed and sample labels are checked against the chain of custody form (COC). If a sample is missing, mislabeled, or in poor condition this is promptly reported to the client. NEON samples require documentation of sample receipt in an electronic form provided by NEON staff. See Section 9.1.4.

2.2 Algal samples may be subsampled for soft algae analysis and diatom analysis. resulting in two separate samples for storage.

2.3 The diatom subsample is digested in nitric acid in the ETHOS UP microwave digester to remove all organic matter prior to storage in scintillation vials. This subsample of cleaned diatom cells is later used to make mounted diatom slides for analysis, see GLEC SOP ALG 10002A.

2.4 This SOP draws from protocols outlined in the following documents:

2.4.1 The National Rivers and Streams Assessment 2023-2024 Laboratory Operations Manual;

2.4.2 “The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program,” Report No. 02-06, the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section; and

2.4.3 Acker 2016 “Identification and enumeration of algae in phytoplankton samples”.

III. DEFINITIONS

3.1 Diatom algae – Diatoms are algae with distinctive, transparent cell walls made of silicon dioxide hydrated with a small amount of water.

3.2 Deionized (DI) water – Water that has had its mineral ions removed. It is produced by purifying tap water by reverse osmosis (RO) followed by passing it through carbon and de-ionization cartridges. It is a physical process using ion exchange resins, which bind to and filter out the mineral salts from water.

3.3 Digestion – Refers to the solubilization of organic material by strong acid oxidation, which may be aided by pressure and heat generated by microwaves.

3.4 Safety Data Sheet (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, flammability, and reactivity, including how it should be stored, handled, and disposed.

3.5 Soft algae – Soft-bodied, non-diatom algae.

3.6 Macroalgae - 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).

3.7 Subsample - Portion of the sample obtained by randomly extracting a volume from the composite sample collected by the field crew.

3.8 Microwave digester/digestion – The ETHOS UP is a microwave system which uses contactless microwave irradiation to heat material. This allows organic material in diatom samples to be digested, leaving the silicon dioxide valves in place.

IV. INTERFERENCES AND CAUTIONS

4.1 Samples preserved in alcohol should never be treated with nitric acid due to the risk of an explosive reaction. If you believe a sample may have been mistakenly preserved in ethanol, please set it aside and inform the lab manager.

4.2 The nitric acid digestion in the microwave digester may release corrosive fumes. The discharge vent hose should be secured in a functioning fume hood which should be running during active digestion.

4.3 Do not homogenize soft algae subsamples enough to break up microscopic colonies. This will make the soft algae identification more difficult.

V. EQUIPMENT AND SUPPLIES

5.1 Carboy for DI

5.2 Chemical fume hood

5.3 Erlenmeyer flask – 1L or 500 mL, to pour DI into beakers

5.4 ETHOS UP microwave digester - with nylon digestion tubes, with valves and screw caps, and carousel

5.5 Forceps

5.6 Lab coat

5.7 Laboratory labeling tape

5.8 Laboratory trays

5.9 Nitrile gloves

5.10 Permanent markers and pens

5.11 Project specific sample log

5.12 250 mL wash bottle

5.13 Safety glasses

5.14 Syphon - to remove supernatant when rinsing cleaned diatom samples

5.15 Tape - electrical or similar

5.16 Watch glasses, aluminum foil, or parafilm to cover cleaned diatom samples during rinsing period

5.17 5 mL pipette and tips - one tip per sample

5.18 20 mL scintillation vials

5.19 150 mL beakers, - labeled with sample ID, one per sample plus extras for reagents/water

VI. REAGENTS AND STANDARDS

6.1 Reagent Water – DI water

6.2 Reagents –

6.2.1 Concentrated nitric acid (HNO_3)

6.2.2 70% ethanol

6.2.3 10% hydrochloric acid (HCl)

6.2.4 Glutaraldehyde

VII. SAMPLE RECEIPT

7.1 Sample Receipt (Workflow Chart for Sample Receipt is provided below in Figure 1).

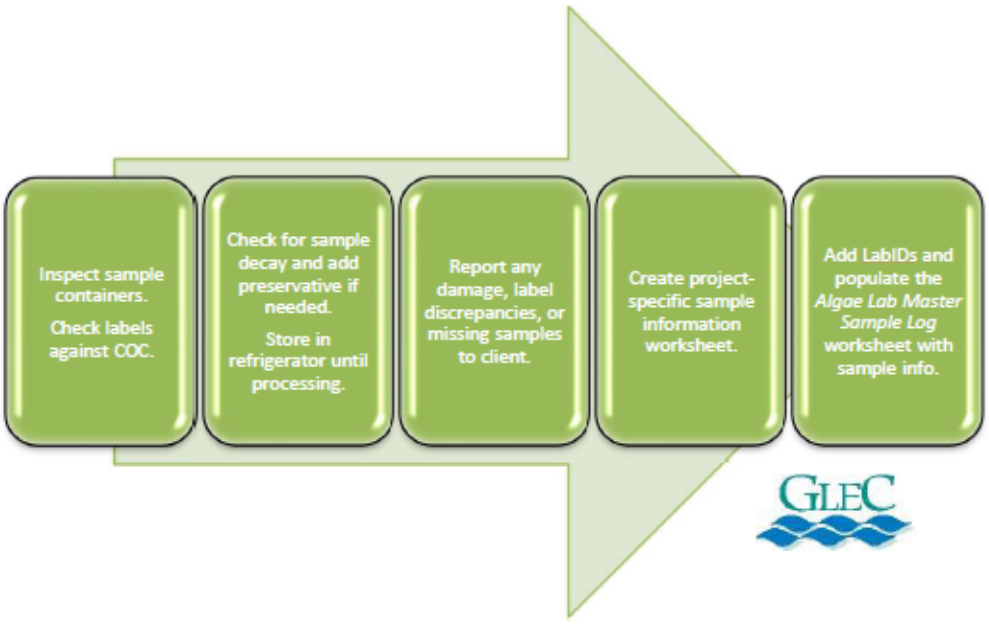


Figure 1. Workflow Chart for Sample Receipt

7.1.1 Chain of custody form (COC), listing a unique sample ID for each sample shipped, should be included with each shipment to GLEC.

Note: NEON samples are preserved in glutaraldehyde.

7.1.2 Upon sample arrival at GLEC, evaluate the condition of each sample container, checking for cracks, leakage, etc. Cross-check the sample information on the sample containers against the sample information listed on the COC form(s). Smell the sample to determine if any decay is

occurring. If decay is detected, add more of the original preservative to all original samples. This addition should be recorded in the sample information sheet. NEON sample receipt will be documented in an electronic receipt form provided by NEON staff.

7.1.3 In the event of any container damage, label discrepancies, and/or missing samples, the client should be contacted before processing the samples. For NEON samples, the technician should "Reply All" to the shipping notification email.

7.1.4 Inform client of sample receipt. For NEON samples, this includes completing an electronic receipt form provided by NEON staff. When completed, inform the lab manager and they will upload this receipt form to the NEON Data Portal.

7.1.5 All Sample information should be entered into a project-specific Excel spreadsheet for tracking and protocol documentation. Use the bar code reader to directly populate the project-specific Excel worksheet.

7.2 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. Cleaned diatom subsamples may be kept at room temperature.

7.3 Samples with live algae should be refrigerated, kept in the dark and processed or preserved within 2 days of receipt.

VIII. QUALITY CONTROL

8.1 Clean glassware, nylon digestion tubes and valves/screw caps, Nalgene bottles, 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. Soak and rinse forceps or other utensils used while processing samples. Rinse siphon tube with DI water between samples. If discoloration is noted on the nylon digestion tubes, they may be cleaned with nitric acid.

8.2 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.

IX. PROCEDURE

9.1 Subsampling, Archiving, and Shipping

9.1.1 Check that each sample is intact and smell the sample to determine if any decay is occurring. If decay is detected, add more of the appropriate preservative to all the original samples and note this addition on the data sheets.

9.1.2 Measure the sample volume and note any deviations from the expected volume.

9.1.3 Multiple subsamples may be taken from the original algal sample. The following steps outline the process of subsampling for both soft algae analysis and diatom analysis. All steps may not be required for all contracts. A workflow chart for the archiving of NEON samples is provided in Figure 2 below.

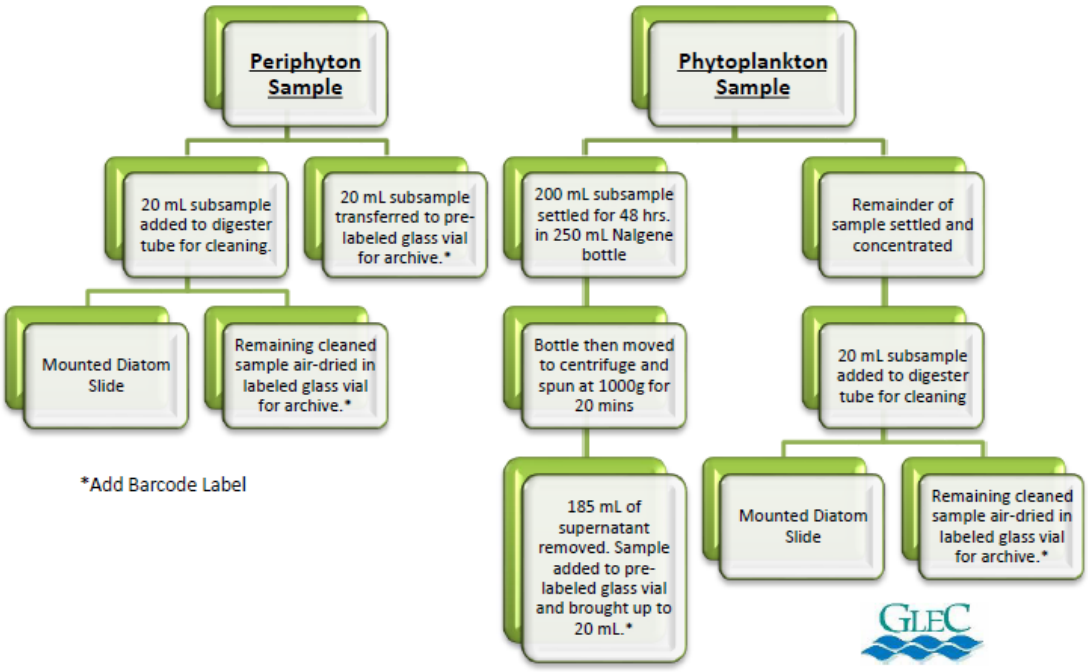


Figure 1. NEON Sample Archiving Workflow

9.1.3.1 If samples are to be digested for diatom slides, start a Diatom Slide Preparation Worksheet including the date of digestion, number of samples, amount digested (generally 20 mL, note deviations), and initials of technician.

9.1.3.2 Using preprinted labels, label 20 mL scintillation vials for the soft algae subsamples. If applicable, label corresponding scintillation vials for the cleaned diatom subsample and set aside. Using lab tape, mark 150 mL beakers with the sample ID. These beakers will be used for digestion of the diatom subsamples. If archiving NEON phytoplankton samples, use label tape to mark clean 250 mL Nalgene bottles for settling and centrifugation.

9.1.3.2.1 NEON archive subsamples should be labeled according to the NEON protocol outline in "Exhibit A_SOW Macroalgae Taxonomy and Algae Archiving" pages 4-5

For all subsampling, suspend and homogenize the algal sample by inverting or swirling the original sample immediately before removing sample.

9.1.3.3 Soft Algae

9.1.3.3.1 For Periphyton Samples: Using a 5 mL pipette with a clean tip, before the material settles again, transfer a 10 mL aliquot to the pre-labeled 20 mL glass sample vial. Invert the sample again to homogenize and remove another 10 mL of sample. This soft algae subsample will be kept in a sample refrigerator, at 4ºC until taxonomic analysis.

9.1.3.3.2 For Phytoplankton Samples: Decant 200 mL of homogenized sample into the pre-labeled 250 mL Nalgene bottles. Allow the samples to sit, unmoved for 48 hours to allow algal cells to settle to the bottom. Carefully transfer these settled samples to the centrifuge. Centrifuge at 1000g for 20 minutes.

9.1.3.4 Diatoms

9.1.3.4.1 For Periphyton Samples: Invert the original sample to homogenize and, before the material settles again, transfer a 10 mL aliquot to a nylon ETHOS UP digester tube using a 5 mL pipette

with a clean tip. Invert the original sample again to homogenize and remove another 10 mL of sample to the tube. Proceed to Microwave Digestion Procedure outlined in 11.2.

9.1.3.4.2 For Phytoplankton Samples: Allow the remainder of the phytoplankton sample (800 mL) to settle, undisturbed, for 48 hours. Concentrate the sample ~10X by removing an appropriate volume of the supernatant. Invert the remaining sample to homogenize and, before the material settles again, transfer a 10 mL aliquot to a nylon ETHOS UP digester tube using a clean 5 mL pipette tip. Invert the original sample again to homogenize and remove another 10 mL of sample to the tube. Proceed to Microwave Digestion Procedure outlined in 11.2.

9.1.3.4.3 Digested diatom samples can be stored at room temperature. Archive diatom samples are to be air dried for long-term storage. After a diatom slide been made and QC reviewed for quality, the cleaned diatom samples are prepared for air drying. Allow samples to settle, undisturbed, in the 20 mL glass vials, for 48 hrs. After this time, carefully remove ≥ 15 mL of the overlying water using a 5 mL pipette, with a clean tip, without disturbing the diatom cells settled on the bottom. Cover, leaving cap slightly ajar to allow the remaining water to dehydrate.

9.1.4 Barcode labels should be added to all NEON archive samples. Follow the labeling protocol outlined in “Exhibit A_SOW_Macroalgae Taxonomy and Algae Archiving” pages 4-5.

9.1.5 NEON Sample Return: All samples prepared for archiving will be shipped to the NEON Biorepository at Arizona State University following the guidelines in Appendix 1 in “Exhibit A_SOW_Macroalgae Taxonomy and Algae Archiving” pages 4-5. Leftover sample may be discarded after archive samples have been received and approved by NEON.

9.2 Diatom Microwave Digestion

9.2.1 Load samples into their respective tubes.

9.2.2 Calculate the necessary amount of nitric acid for the run (10 mL per sample) and carefully pour this volume into a labelled glass beaker in the hood.

9.2.3 Using the 5 mL pipette and a single pipette tip, add 10 mL nitric acid to each nylon tube. Let sit with tubes open for 45 minutes to 'predigest' in fume hood. Cap each of the sample tubes with the two-part screw on caps.

9.2.4 After pre-digestion, set the carousel with location number one at the front. Close the ETHOS UP door and load the 'diatom digestion method.' Click the green start arrow on the home screen to begin the digestion. Enter the number of samples in the carousel (1-32 out of 44).

9.2.5 Ensure that the vent tube is secured inside the fume hood, which should remain on for the duration of the digestion protocol.

9.2.6 Once the protocol is complete, the door will remain locked until the vials are fully cooled to less than 80°C.

9.3 Diatom Rinsing and Sample Transfer

9.3.1 Label a 150 mL glass beaker for each sample digested and place on a black lunch tray inside the fume hood, which should be on for all subsequent steps.

9.3.2 Open the ETHOS UP, remove the carousel, and set in the fume hood.

9.3.3 Decant each sample into the appropriate labelled beaker. Rinse the tube several times with a DI filled rinse bottle, pouring the rinsate into the beaker. Visually check that all sediment has been rinsed out of the tube into the beaker.

9.3.4 Once all samples are decanted, carefully fill the beakers with DI water. Check 'DI Top Off' box on the Worksheet.

9.3.5 Diatom samples must be allowed to settle at least eight hours between each rinse. Set the tray to the side of the laboratory sink and cover samples with aluminum foil, parafilm, or a watch glass to avoid contamination.

9.3.6 After settling, syphon off the supernatant from each beaker. Rinse the syphon tip with tap water between each sample. Do not remove more supernatant than can be removed comfortably without disturbing the settled diatom layer.

9.3.7 Once the supernatant has been removed from all samples, refill the beakers with DI water and note the date, time, and initials of the rinse on the Worksheet. Repeat syphoning and rinsing six times, or until the overlaying water is approximately pH neutral (6-8 using pH paper). If more than 10 mL of nitric acid was used in the digestion, additional rinses may be necessary. If less than 10 mL of nitric acid was used in the digestion, rinsing could be less than six times.

9.3.8 After the final rinse and settling period, prepare 20 mL glass scintillation vials labeled for each sample and syphon off as much supernatant as possible without disturbing diatom layer.

9.3.9 Gently swirl each beaker to resuspend the diatoms in the remaining DI water and quickly pour this slurry into the appropriate vial. Rinse the sides and bottom of the beaker and pour this into the vial. Be conservative with the DI water, as the glass vials cannot be overfilled, total volume should be 20 mL or less.

9.3.10 After all the sample contents are transferred from the 150 mL beaker to the 20 mL vial, bring the final volume of the glass vial up to 20 mL with DI water. The vials hold 20 mL at the shoulder.

X. DATA ANALYSIS AND CALCULATIONS

Not Applicable.

XI. EQUIPMENT MAINTENANCE

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

XII. REFERENCES

12.1 Acker, F. 2016. Identification and enumeration of algae in phytoplankton samples. Procedure No. P-13-52. 8 pages.

12.2 EPA-841-B-07-010. National Rivers and Streams Assessment: Laboratory Methods Manual, Nov. 2009. http://www.epa.gov/sites/production/files/2013-11/documents/nrsa200809_labmethodsmanual_20091229_2_web.pdf

12.3 ETHOS UP User Manual. (2018). Milestone.

12.4 GLEC SOP ALG 10002A. Permanent Diatom Slide Preparation.

12.5 Report No. 02-06 the Academy of Natural Sciences, Patrick Center for Environmental Research-Phycology Section. "The protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program," May 2012. http://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf

12.6 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.

STANDARD OPERATING PROCEDURE FOR MACROALGAE IDENTIFICATION AND DATA SUBMISSION-ABBREVIATED

ALG 10006A

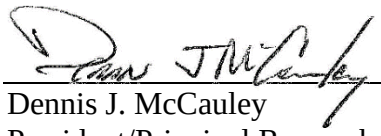
Method Reference: This method is based on Stancheva et al. 2015

October 11, 2024

Great Lakes Environmental Center, Inc.
(GLEC)

10/11/2024

Julianne Heinlein — Date
Technical Approver



10/11/2024

Dennis J. McCauley — Date
President/Principal Research Scientist

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Jennifer Hansen — Date
GLEC Quality Assurance Officer

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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. Gridded 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, spread all of the biomass collected from the sample, separated by taxa, onto the gridded Petri dish. Use the grid to estimate each species' proportion of the total macroalgae biomass, their relative abundance. This should include large colonial diatoms if present. See Figure 1 below taken from Stancheva et al. (2015).

7.2.7.1 Count the number of grid squares occupied by all algae combined, the total squares (TS), and the number of grid squares occupied by each individual taxon, the species squares (SS). The relative abundance (RA), or percent composition, for each taxon is calculated as follows: $RA = \left({}^{SS}/_{TS}\right)100$.

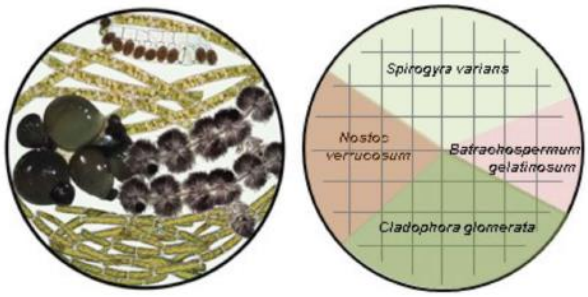

Figure 1. Illustration of Macroalgae Relative Abundance Estimate - Macroalgal sample placed in a gridded Petri dish to estimate the proportions of species. In this case: Cladophora glomerata (31%), Nostoc verrucosum (24%), Batrachospermum gelatinosum (15%), and Spirogyra varians (30%). Taken from Stancheva et al. (2015)

7.3 All material should be returned to the original sample container for long-term storage.

7.3.1 NEON Sample Return: All samples prepared for archiving will be shipped to the NEON Biorepository at Arizona State University following the guidelines in Appendix 1 in "Exhibit A_SOW_Macroalgae Taxonomy and Algae Archiving" pages 4-5.

7.3.1.1 Unidentified NEON macroalgae subsamples created will be labeled with the sample ID they had upon arrival. Identified samples shall be stored in labeled vials and must include the original NEON program sample ID. This ID contains information about the site, date, and type of field sample.

7.3.1.1.1 The macroalgae format is SITE.DATE.morphospeciesID.location, example "ARIK.20180610.macroalgae1.Q3".

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.

XII. TAXONOMIC REFERENCES

12.1 https://www.algaebase.org/

12.2 Carty, S. Freshwater Dinoflagellates of North America. Cornell University Press, Ithaca.

12.3 Ciugulea, I. and R.E. Triemer. 2010. A Color Atlas of Photosynthetic Euglenoids. Michigan State University Press, East Lansing, MI. 204 p.

12.4 Dillard, G.E. 1989. Bibliotheca Phycologica. Band 81. Freshwater Algae of the Southeastern United States. Part 1. Chlorophyceae: Volvocales, Tetrasporales and Chlorococcales. J. Cramer, Stuttgart.

12.5 Dillard, G.E. 1989a. Bibliotheca Phycologica. Band 83. Freshwater Algae of the Southeastern United States. Part 2. Chlorophyceae: Ulotrichales, Microsporales, Cylindrocapsales, Sphaeropleales, Chaetophorales, Cladophorales, Schizogoniales, Siphonales and Oedogoniales. J. Cramer, Stuttgart.

12.6 Dillard, G.E. 1990. Bibliotheca Phycologica. Band 85. Freshwater Algae of the Southeastern United States. Part 3. Chlorophyceae: Zynematales: Zygnemataceae, Mesotaeniaceae and Desmidiaceae (Section 1). J. Cramer, Stuttgart.

12.7 Dillard, G.E. 1991. Bibliotheca Phycologica. Band 89. Freshwater Algae of the Southeastern United States. Part 4. Chlorophyceae: Zygnematales: Desmidiaceae (Section 2). J. Cramer, Stuttgart.

12.8 Dillard, G.E. 1991a. Bibliotheca Phycologica. Band 90. Freshwater Algae of the Southeastern United States. Part 5. Chlorophyceae: Zygnematales: Desmidiaceae (Section 3). J. Cramer, Stuttgart.

12.9 Dillard, G.E. 1993. Bibliotheca Phycologica. Band 93. Freshwater Algae of the Southeastern United States. Part 6. Chlorophyceae: Zygnematales: Desmidiaceae (Section 4). J. Cramer, Stuttgart.

12.10 Dillard, G.E. 2000. Bibliotheca Phycologica. Band 106. Freshwater Algae of the Southeastern United States. Part 7. Pigmented Euglenophyceae. J. Cramer, Stuttgart.

12.11 Dillard, G.E. 2007. Bibliotheca Phycologica. Band 112. Freshwater Algae of the Southeastern United States. Part 8. Chrysophyceae, Xanthophyceae, Raphidophyceae, Crysophyceae and Dinophyceae. J. Cramer, Stuttgart.

12.12 Ettl, H. 1978. Xanthophyceae. In Ettl, H., J. Gerloff & H. Heynig (eds), Süsswasserflora von Mitteleuropa 2. Gustav Fischer, Stuttgart.

12.13 Ettl, H. 1983. Phytomonadina. In Ettl, H., J. Gerloff & H. Heynig (eds), Süsswasserflora von Mitteleuropa 1. Gustav Fischer, Stuttgart.

12.14 Ettl, H., and G. Gärtner 1988. Chlorophyta II: Tetrsporales, Chlorococcales, Gloeodendrales. In Ettl, H., J. Gerloff, H. Heynig, and D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa 10. Gustav Fischer, Stuttgart.

12.15 Hindák, F. 2008. Colour Atlas of Cyanophytes. VEDA, Bratislava.

12.16 Heynig & D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa 16. Gustav Fischer, Stuttgart.

12.17 Komárek, J., B. Fott and G. Huber-Pestalozzi. 1983. Chlorophyceae (Grünalgen). Ordnung: Chlorococcales. Binnengewasser. Phytoplankton des Susswasser. Schweizerbart, Stuttgart.

12.18 Komárek, J., and K. Anagnostidis 1999. Cyanoprokaryota: Chroococcales. In Ettl, H., G. Gärtner, H. Heynig & D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa 19/1. Gustav Fischer, Stuttgart.

12.19 Komárek, J., and K. Anagnostidis 2005. Cyanoprokaryota: Oscillatoriales. In Büdel, B., G. Gärtner, L. Krienitz & M. Schagerl (eds), Süsswasserflora von Mitteleuropa 19/2. Elsevier, München.

12.20 Komárek, J. 2013. Cyanoprokaryota: Heterocytous Genera. In Büdel, B., G. Gärtner, L. Krienitz & M. Schagerl (eds), Süsswasserflora von Mitteleuropa 19/3. Springer, Berlin.

12.21 Kristiansen, J., and H. R. Preisig. 2007. Chrysophyte and Haptophyte algae. Part 2: Synurophyceae. In Büdel, B., G. Gärtner, L. Krienitz, H. R. Preisig & M. Schagerl, (eds), Süsswasserflora von Mitteleuropa 2/2. Springer-Verlag Berlin Heidelberg.

12.22 Ling, H.U. and P.A. Tyler. 2000. Bibliotheca Phycologica, Band 105. Australian Freshwater Algae (exclusive of diatoms). J. Cramer, Stuttgart.

12.23 Moestrup, Ø and A. Calado. 2018. Dinophyceae. In Ettl, H., J. Gerloff & H. Heynig (eds), Süsswasserflora von Mitteleuropa 6. Gustav Fischer, Stuttgart.

12.24 Prescott, G. W. 1951. Algae of the Western Great Lakes Area. WM.C. Brown Publishers, Dubuque, Iowa, 977 pp.

12.25 Prescott, G. W., C. E. M Bicudo, and W. C. Vinyard. 1982. A Synopsis of North American Desmids. Part II. Desmidiaceae: Placodermae, Section 4. University of Nebraska Press, Lincoln.

12.26 Prescott, G. W., H. T. Croasdale, and W. C. Vinyard. 1975. A Synopsis of North American Desmids. Part II. Desmidiaceae: Placodermae, Section 1. University of Nebraska Press, Lincoln.

12.27 Prescott, G. W., H. T. Croasdale, and W. C. Vinyard. 1977. A Synopsis of North American Desmids. Part II. Desmidiaceae: Placodermae, Section 2. University of Nebraska Press, Lincoln.

12.28 Prescott, G. W., H. T. Croasdale, W. C. Vinyard, and C. E. M. Bicudo. 1981. A Synopsis of North American Desmids. Part II. Desmidiaceae: Placodermae, Section 3. University of Nebraska Press, Lincoln.

12.29 Reith, A.1980. Xanthophyceae. In Ettl, H., J. Gerloff & H. Heynig (eds), Süsswasserflora von Mitteleuropa 2. Gustav Fischer, Stuttgart.

12.30 Sheath, R. G. 2003. Red algae. In Wehr, J. D. & R. G. Sheath (eds), Freshwater Algae of North America: Ecology and Classification. Academic Press, San Diego, California: 197-221.

12.31 Skaloud, P, F. Rindi, C. Boedecker, and F. Leliaert. 2018. Chlorophyta: Ulvophyceae. In Ettl, H., J. Gerloff & H. Heynig (eds), Süsswasserflora von Mitteleuropa 13. Gustav Fischer, Stuttgart.

12.32 Smith, G. M. 1950. The Fresh-Water Algae of the United States. 2nd ed. McGraw-Hill Book Co., New York.Stancheva, R., C. Fuller & R. G. Sheath. 2014. Soft-Bodied Stream Algae of California.

12.33 Starmach, K. 1985. Chrysophyceae und Haptophyceae. In Ettl, H., J. Gerloff, H. Heynig & D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa 1. Gustav Fischer, Sttutgart.

12.34 Strunecký O, Ivanova AP, Mareš J. An updated classification of cyanobacterial orders and families based on phylogenomic and polyphasic analysis. J Phycol. 2023 Feb;59(1):12-51. doi: 10.1111/jpy.13304. Epub 2023 Jan 13. Erratum in: J Phycol. 2023 Jun;59(3):635. doi: 10.1111/jpy.13355. PMID: 36443823.

12.35 Wehr, J. D., R. G. Sheath, and J. P. Kociolek (eds). 2015. Freshwater Algae of North America: Ecology and Classification, 2nd Ed. Elsevier Academic Press, San Diego, California.

12.36 Wołowski, K. & Hindák, F. 2008. Atlas of Euglenophytes. VEDA, Bratislava.