



**SWAMP Bioassessment Procedures 2015**

# Standard Operating Procedures for Laboratory Processing, Identification, and Enumeration of Stream Algae

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## LIST OF ACRONYMS AND ABBREVIATIONS

Term	Definition
CalPAL	SWAMP California Primary Algae Laboratory of the State Water Board
CEDEN	California Environmental Data Exchange Network
COC	Chain of Custody
DI water	DI water - Deionized Water
DIC	Differential Interference Contrast
DMT	Data Management Team
ID	Identification
MQOs	Measurement Quality Objectives
MSDS	Material Safety Data Sheets
NCE	Natural Counting Entity
PTA	Percent Taxonomic Agreement
QA	Quality Assurance
QC	Quality Control
Sample ID	Unique sample name
SBA	Soft-Bodied Algae
SOP	Standard Operating Procedures
STE	Standard Taxonomic Effort
SWAMP	Surface Water Ambient Monitoring Program

# REQUIREMENTS AND RECOMMENDATIONS FOR SWAMP FUNDED PROJECTS

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
1.1	Laboratory Practices	Taxonomist Qualifications	<p>The laboratory must have at least one taxonomist (preferably two) who meets the minimum qualifications specified in Section 1.1.</p> <p>Maintain current documentation of taxonomist qualifications, and be prepared to submit these for review upon request.</p>	None
1.2	Laboratory Practices	Laboratory Technician Qualifications	<p>Laboratory technicians must meet the minimum qualifications specified in Section 1.2.</p> <p>Laboratories must maintain current training documentation of all laboratory technicians, and be prepared to submit these for review upon request.</p>	None
1.3	Laboratory Practices	Taxonomic Literature	Remain current with taxonomic literature related to local algal flora.	List of algal taxonomic resources is included in Appendix I: References
1.4	Laboratory Practices	Taxonomic Nomenclature	<p>Use the taxon names compiled in the SWAMP Algae Master Taxa list.</p> <p>Newly reported species must be well documented and submitted for harmonization.</p> <p>Taxon names of newly reported species must be approved prior to reporting.</p>	None

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
1.5.1	Laboratory Practices	Photographic Documentation of Newly Reported Taxa	Collect high-quality photomicrographs for each newly reported species submitted to the Algae Master Taxa list.	Refer to Section 5.4 and Appendix H
1.5.2	Laboratory Practices	Photographic Documentation of Previously Reported Taxa	None	Collect representative photomicrographs of each SBA and diatom taxon identified in the sample
1.5.3	Laboratory Practices	General Requirements for Photographic Documentation	<p>Photomicrograph documentation must have the following:</p> <p>Scale bar in the lower right corner of the image measuring 10, 20 or 50 <math>\mu\text{m}</math> proportional to the size of the algae and magnification used;</p> <p>Be saved in TIFF format using the maximum resolution afforded by the equipment in use (minimum of 300 dpi);</p> <p>Each photo should have a filename consisting of the following elements in the order indicated: SWAMP Sample ID, Sampling date (MM/DD/YYYY), Species ID, magnification for objective (i.e. 40x).</p>	Store all photomicrographs on a high-capacity internal hard drive of the laboratory computer and periodically backed up onto an external hard drive.
1.6.1	Laboratory Practices	Standard Taxonomic Effort for SBA	SBA specimens to be identified to species level, or the lowest taxonomic level possible	Identify each SBA to species level or lower. If species identification is not possible due to insufficient taxonomic vegetative or reproductive data, identify the specimen to the lowest taxonomic level possible, such as genus or above.

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
1.6.2	Laboratory Practices	Standard Taxonomic Effort for Diatoms	Diatom specimens to be identified to species level, or the lowest taxonomic level possible	Identify each diatom to species level or lower. If species identification is not possible due to insufficient taxonomic data, identify the specimen to the lowest taxonomic level possible, such as genus or above.
1.7	Laboratory Practices	External Taxonomic Harmonization Process	Submit all newly reported species identifications for taxonomic harmonization prior to reporting.	SWAMP recommends harmonization of the entire dataset (including results from previously reported species), but does not currently require this due to resource limitations.
1.8	Laboratory Practices	Training	Laboratories must have internal procedures for executing and documenting the training of laboratory technicians and taxonomists in the use of these procedures.	Documentation of training should include demonstration of performance in the procedures.
1.9	Laboratory Practices	General Taxonomic Laboratory Practices	None	None
2.1	Laboratory Sample Receipt	Sample Receipt	Confirm that the sample labels match the chain of custody (COC) forms and all samples are accounted for.  Retain copies of the COCs.	None



Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
2.2.1	Laboratory Sample Receipt	SBA Qualitative Sample Integrity Check	<p>Confirm the sample is received in a 100 mL Whirl-Pak® bag.</p> <p>Confirm the sample is cool (4 °C) upon receipt.</p> <p>Confirm sample has not been frozen.</p> <p>Note evidence of freezing on the COC. Confirm sample did not leak prior to receipt. Note evidence of leaking on the COC.</p> <p>Confirm the sample has been received within 2 weeks of collection.</p>	None
2.2.2	Laboratory Sample Receipt	SBA Quantitative Sample Integrity Check	<p>Confirm that the SBA quantitative sample has been preserved. If the sample received is unpreserved, it must be preserved ASAP within 4 days of collection.</p> <p>If the sample is preserved following receipt, record the volume of the unpreserved sample, amount of glutaraldehyde added, and date and time of preservation on the COC.</p>	Inspect the volume in the sample vial. Vials received with less than 50 mL of preserved sample may indicate the sample was not preserved or had leaked during transport.
2.2.3	Laboratory Sample Receipt	Diatom Quantitative Sample Integrity Check	<p>Confirm the samples received are preserved in the field with 1% formalin.</p>	Inspect the volume in the sample vial. Vials with less than 50 mL of preserved sample may indicate the sample was not preserved or had leaked during transport.
2.2.4	Laboratory Sample Receipt	Receipt of Broken Sample Vials	<p>Transfer leaking sample to a new 50 mL plastic centrifuge tube labeled with the sample information.</p> <p>Measure and record the remaining sample volume. Document the sample condition.</p> <p>Add additional preservative and note volume added on COC.</p>	None

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
3.1.1	Sample Preparation	SBA Qualitative Sample Preparation	None	Archiving of the SBA Qualitative sample should be conducted as soon as possible following completion of the taxonomic analysis.
3.1.2	Sample Preparation	SBA Quantitative Sample Preparation – Macroalgal Fraction	<p>Confirm the absence of macroalgae using the dissecting microscope before proceeding with microalgae preparation.</p> <p>Determine the biovolume of macroalgae by water displacement.</p>	None
3.1.3	Sample Preparation	SBA Quantitative Sample Preparation – Microalgal Fraction	Homogenize the microalgal fraction of the SBA Quantitative sample by gently but thoroughly inverting the 50 mL centrifuge tube several times.	None
3.1.4	Sample Preparation	SBA Semi-permanent Slide Preparation of Quantitative Microalgal Fraction	Confirm the prepared slide contains a random distribution of microalgae that is sufficiently dense for species identification and enumeration.	Gently tap on the cover slip to reduce the algae clumping and air bubbles, if present.
3.2.1	Sample Preparation	Cleaning of Diatom Samples: Nitric Acid Method	Diatom Quantitative samples are preserved in formalin, so they must be handled carefully.	None
3.2.2	Sample Preparation	Cleaning of Diatom Samples: Hydrogen Peroxide Method	Specific handling and disposal procedures must be in place for handling hydrogen peroxide and potassium dichromate.	None

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
3.2.3	Sample Preparation	Slide Preparation of Diatom Samples	Confirm the prepared slide contains a random distribution of diatoms that is sufficiently dense for conducting identification and enumeration procedures.	Add 10% HCl to the cleaned diatom suspension to achieve a more even distribution of diatom valves on the coverslip.
4.1.1	Identification and Enumeration Analysis of Algae	SBA Qualitative Sample Analysis	<p>Record all macroalgal taxa identified in the SBA qualitative sample in the <i>ID Datasheet for SBA Sample under the heading Qualitative sample – list of taxa</i></p> <p>Take sufficient high-quality photomicrographs of all newly recorded species to support harmonization of results.</p>	<p>Collect photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification.</p> <p>When reproducing filaments of zygnematalean algae are observed, but completely matured zygospores/aplanospores are not available, further incubation under nutrient stress facilitates completion of sexual or asexual reproduction. The resulting mature zygospores (or akinetes, aplanospores) can provide the taxonomist with the additional information needed to identify the species.</p>
4.1.2	Identification and Enumeration Analysis of Algae	SBA Quantitative Sample Analysis - Macroalgal Fraction	Record the fraction represented by non-algal matter on the <i>ID Datasheet for SBA Sample Heading: non-algal matter xx %</i> .	Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification.

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
4.1.2 (cont)	Identification and Enumeration Analysis of Algae	SBA Quantitative Sample Analysis - Macroalgal Fraction	<p>Record the identification for each macroalgal taxon identified and the corresponding proportion of each in the <i>ID Datasheet for SBA Sample-Heading: Macroalgae taxon ID; Proportion of each taxon (%)</i>.</p> <p>Enumerate 100 NCEs of epiphytic SBA alga attached to the surface of the macroalgae. Record each epiphytic algae taxa identified and the corresponding number of NCEs enumerated on the <i>ID Datasheet for SBA Sample-Heading: Epiphyte taxon ID; #NCE</i>.</p> <p>Take sufficient high-quality photomicrographs of all newly recorded species to support harmonization of results.</p>	Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification.
4.1.3	Identification and Enumeration Analysis of Algae	SBA Quantitative Sample Analysis - Microalgal Fraction	<p>Record any additional dilution or concentration performed on the sample in the <i>ID Datasheet for SBA Sample-Heading: Quantitative Sample-Microalgal fraction-sample volume after additional dilution/concentration: xx mL; dilution factor</i></p> <p>Identify and enumerate 300 SBA NCEs across a known number of horizontal optical transects.</p>	<p>Ensure that the volume of the drop is not so large that it creates the formation of bubbles or causes the cover slip to float.</p> <p>Avoid having too much or too little material on the slide.</p> <p>Gentle tapping on the cover slip or spread clumps apart with a pair of dissecting needles will reduce clumping of algae.</p>

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
4.1.3 (cont)	Identification and Enumeration Analysis of Algae	SBA Quantitative Sample Analysis - Microalgal Fraction	<p>Record each microalgal SBA species identified and the corresponding number of NCEs enumerated on the <i>ID Datasheet for SBA Sample-Heading: Microalgal taxon ID; #NCE</i>).</p> <p>Record the number of transects traversed in <i>ID Datasheet for SBA Sample-Heading: Microalgal fraction-number of horizontal transects counted: xx</i>.</p> <p>Determine the appropriate geometric model for each microalgal species identified and perform microscopic measurements of the cell dimensions for each. Record measurements on the <i>ID Datasheet for SBA Sample-Heading: Cell diameter (<math>\mu\text{m}</math>); Cell/NCE length (<math>\mu\text{m}</math>); Cell Depth (<math>\mu\text{m}</math>); Total number of cells; Total filament length(<math>\mu\text{m}</math>).</i></p> <p>Take sufficient high-quality photomicrographs of all newly recorded species to support harmonization of results.</p>	Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification.
4.1.4.1	Identification and Enumeration Analysis of Algae	Biovolume Calculations: SBA Quantitative Sample-Macroalgal Fraction	Calculate the biovolume of each macroalgal taxon using the formulas in Section 4.1.4.1.	None

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
4.1.4.2	Identification and Enumeration Analysis of Algae	Biovolume Calculations: SBA Quantitative Sample-Microalgal Fraction	Calculate the biovolume of each microalgal taxon using the formulas in Section 4.1.4.2.	None
4.2	Identification and Enumeration Analysis of Algae	Enumeration and Identification Analysis of Diatoms	<p>Identify and enumerate 600 diatom valves across a known length of horizontal optical transects.</p> <p>Partial valves are defined as having more than 50% of the valve including the central area.</p> <p>Enumerate only complete and partial valves. The valve (both complete and partial) must extend at least halfway into the transect, and must include the center of the valve in the transect.</p> <p>Record each diatom taxon identified and the corresponding number of valves enumerated on the <i>ID Datasheet for Diatom</i> <i>Sample-Heading: Diatom taxon ID; Number of valves</i>).</p> <p>Record the number of transects traversed, starting and ending field of view for each transect in <i>ID Datasheet for Diatom</i> <i>Sample-Heading: Number of transects counted: xx</i>.</p> <p>Take sufficient high-quality photomicrographs of all newly recorded species to support harmonization of results.</p>	<p>If the sample is very sparse, continue counting for 4 hours or until 300 valves are enumerated (whichever comes first), excluding time spent learning new species.</p> <p>Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification.</p>

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
4.3	Identification and Enumeration Analysis of Algae	Sample Labeling and Archiving	All SBA and diatom samples must be retained as voucher specimens until harmonization and reporting of data is complete.	Archives of samples and slides should be retained by the laboratory for two years.
4.3.1	Identification and Enumeration Analysis of Algae	Archiving of SBA – Qualitative Samples	Select a representative subsample that contains all identified macroalgal taxa and fix it with 2% glutaraldehyde final concentration.	None
4.3.2	Identification and Enumeration Analysis of Algae	Archiving of SBA – Quantitative Samples	<p>Slides-microalgal fraction: Seal the cover slip with nail polish, label the microscopic slide by sample ID, collection date (MM/DD/YYYY), and note “microalgae”.</p> <p>Return analyzed macroalgae and archive the macroalgal fraction adding glutaraldehyde to 2% final concentration. Label the tube by sample ID, collection date (MM/DD/YYYY), and note “macroalgae”.</p> <p>Refix the subsample with 2% glutaraldehyde final concentration and keep separately from original sample for reference purposes. Label it by sample ID, collection date (MM/DD/YYYY), and note “microalgae”.</p>	None
4.3.3	Identification and Enumeration Analysis of Algae	Archiving of Diatoms	<p>Label each slide by sample ID, collection date (MM/DD/YYYY), and note “diatoms”.</p> <p>Fix remaining cleaned diatom material with ethanol to 50% final concentration. Label each vial by sample ID, collection date (MM/DD/YYYY), and note “diatoms”.</p>	None

Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
5.1	Quality Assurance and Quality Control	Laboratory Quality Control	None	None
5.2	Quality Assurance and Quality Control	Laboratory Quality Assurance	None	None
5.3.1	Quality Assurance and Quality Control	Sample Handling Requirements- Point of Receipt	Confirm samples meet the sample handling requirements in Table 3: Required Sample Conditions for Laboratory Receipt.  Follow corrective actions in Table 4: Required Corrective Actions for Laboratory Receipt.	None
5.3.2	Quality Assurance and Quality Control	Sample Handling Requirements- Archiving	All samples must be archived by the laboratory until results have been harmonized and reported.	None
5.4	Quality Assurance and Quality Control	Photomicrographic Documentation Requirements	Collect high-quality photomicrographic documentation of newly recorded species sufficient to support harmonization of results.	Recommendations for producing high-quality photomicrographs are included in Appendix H.  Collect photomicrographic documentation of previously reported species sufficient to demonstrate the key aspects of vegetative morphology and reproduction used in identification.



Relevant SOP Sections	Section	Element	Description of Requirements	Description of Recommendations
5.5	Quality Assurance and Quality Control	Requirements for External Harmonization of Taxonomic Results	Participate in harmonization of SWAMP algal taxonomy results.	None
6.1	Reporting of Algal Taxonomy Results	Reporting of SBA Results	Taxon names entered onto the ID Datasheet must match the controlled SWAMP Algae Master Taxa list of FinalID names.	None
6.2	Reporting of Algal Taxonomy Results	Reporting of Diatom Results	Taxon names entered onto the ID Datasheet must match the controlled SWAMP Algae Master Taxa list of FinalID names.	None
6.3	Reporting of Algal Taxonomy Results	Data Management and Reporting	Submit algae taxonomy data through the Microsoft Excel SWAMP Taxonomy Results template (Taxa Analysis Authorization or Taxa AA form) found on the SWAMP website under the Database Management Systems Templates page.	None

# INTRODUCTION

This standard operating procedure (SOP) is applicable to the analysis of benthic soft-bodied algae (SBA) and diatoms collected using SWAMP standard operating procedures for collection of field data for ambient bioassessments of California wadeable streams: benthic macroinvertebrates, algae and physical habitat (Ode et al., 2015). It describes the staff qualifications, laboratory and taxonomy methods to be used whenever algae stream bioassessment is conducted under the SWAMP program. Since both algal groups, SBA and diatoms, require different laboratory treatment, their separate laboratory processing, identification and enumeration, species documentation, archiving of samples and slides, quality assurance procedures, and data reporting to SWAMP are described. SBA analysis is conducted from two types of samples collected from each stream reach — fresh qualitative and preserved quantitative, resulting in a comprehensive taxa list with corresponding biovolume of algal taxa recorded in the quantitative sample. Diatom analysis from a separate quantitative sample provides a taxa list with the relative abundance of each diatom taxon identified.

## Section 1: Laboratory Practices

This section describes policies for establishing and maintaining necessary infrastructure for processing and identifying soft-bodied algae (SBA) and diatoms. These practices are critical to the production of high-quality algae data.

Laboratories performing identification and enumeration of algal samples using these procedures are required to have the following resources and tools:

- Highly qualified freshwater SBA and diatom taxonomists;
- Highly trained laboratory technicians;
- Research-grade compound microscopes and stereoscopes with capability for attached digital camera;
- Access to up-to-date taxonomic literature;
- A photomicrographic reference collection of SBA and diatom specimens;
- Good general laboratory practices;
- Infrastructure for sample tracking and data management;
- A standard taxonomic effort.

### 1.1 Taxonomist Qualifications

The laboratory must have at least one person (preferably two) with considerable experience in identification and enumeration of all taxonomic groups of stream SBA (called SBA taxonomist) and/or diatoms (called diatom taxonomist, or diatomist). This experience can only be obtained by hands-on algal studies from a variety of freshwater habitats (preferably from streams) with algal identifications corroborated by experts. This experience should also include knowledge of and ability to use the detailed taxonomic references listed at the reference section (Appendix I). In order to remain current with changing algal systematics and nomenclature, the experienced taxonomist(s) must maintain contact with other taxonomists through professional societies and other interactions.

Laboratories must maintain current documentation of taxonomist's qualification, and be prepared to submit these for review upon request.

Taxonomists are responsible for performing identification and enumeration of algae samples, and for training new laboratory personnel in the procedures detailed in this SOP. The experienced taxonomist(s) educate new staff on the specifics of the local algal flora composition, information important to ensure the quality of results.

Algal taxonomists performing analysis for SWAMP projects must meet the following minimum qualifications:

### **Education**

Taxonomists must have at least a Master of Science (MS) degree in botany, ecology, biology or related degree, in addition to one or more of the following:

- Coursework related to plant taxonomy, aquatic ecology or limnology;
- Graduate thesis or undergraduate research projects in algal taxonomy, or ecology of benthic freshwater algae;
- University-level phycology class or SBA taxonomy class (for SBA taxonomist) and diatom taxonomy class (for diatom taxonomist).

### **Experience and Training**

The following experience is required for all taxonomists:

- At least two years of experience identifying freshwater algae, preferably from stream benthos;
- Regularly attend taxonomy training workshops offered by professional meetings;
- New personnel must be trained by more experienced taxonomy staff until the new taxonomist demonstrates the ability to correctly identify local algal species and produces datasets that meet laboratory QA standards.

### **Knowledge and Skills**

The following knowledge and skills are required for all taxonomists:

- Proficiency in the use of appropriate algal taxonomic literature and dichotomous identification keys;
- Current knowledge of the most recent changes in algal taxonomy;

- Navigate the California Online Algae Identification Resource Tools effectively;
- Ability to identify and document algae accurately;
- Experience in the use of light microscopy and digital microphotography for taking high-quality pictures of algal specimens;
- Good record-keeping skills.

## **1.2 Laboratory Technician Qualifications**

Laboratory technicians are responsible for:

- Sample receipt;
- Tracking samples from receipt to archiving;
- Cleaning and preparation of SBA and diatom samples for taxonomic identification and enumeration.

Preparation procedures include subsampling of SBA for macroalgae, measuring the macroalgal fraction volume, concentrating the microalgal subsample, and the cleaning of diatoms and preparing diatom slides for identification by taxonomists.

Laboratory technicians processing algae samples for SWAMP projects must meet the following minimum qualifications:

### **Experience**

- At least two years laboratory experience, with preference given to those who have experience processing bioassessment algal samples;
- Experience in the safe handling of laboratory chemicals.

### **Skills**

- Good record-keeping skills;
- Good hand-eye coordination in sample processing;
- Good skills in quantitative sample processing;
- Ability to process fractions of liquid algal samples with high precision and accuracy;
- Ability to avoid cross-contamination of algal samples;
- Experience in the use of technical equipment and light microscopy for standard algal

specimen preparation techniques, including slide preparation.

Laboratories must maintain current training documentation of all laboratory technicians, and be prepared to submit these for review upon request.

### 1.3 Taxonomic Literature

To properly perform algae identifications, the taxonomist must be up-to-date on the most current taxonomic literature and online resources. Maintaining current knowledge of the taxonomy of local algal flora is critical to ensuring data quality. A number of standard references and online tools have been employed for the identification of freshwater algae across the United States. A list of these resources is included in Appendix I: References.

#### SBA

Although not yet complete, the most comprehensive references for SBA are the 14-volume set, *The Freshwater Flora of Central Europe* (1978-2014), and *The Freshwater Algal Flora of the British Isles* 2<sup>nd</sup> Ed. (John et al., 2011). These references must be used with caution, as not all species are identical to those present in California.

The main floristic works on freshwater algae from the United States are summarized in *Freshwater Algae of North America: Ecology and Classification* 2<sup>nd</sup> Ed. (Wehr et al., 2015). This book notes key references for species identification of SBA from all taxonomic groups documented in the United States, such as Smith (1950), Transeau (1951), Prescott (1951) Prescott et al. (1977-1982), Dillard (1989-2007).

While utilizing these resources, it is important to remember that current knowledge of the freshwater algal diversity of California is incomplete and no one flora is currently available to address all species. Some species have recently been recorded from streams in California (Wehr et al. 2013), or are newly described to science, such as several *Spirogyra* and *Zygnema* species (Stancheva et al. 2012b, 2013), and a new genus of green algae – *Caespitula* (Hall et al., in preparation). Therefore, algal identifications should be done carefully with good knowledge of current literature and local algal flora.

#### Diatoms

The most commonly employed reference for identification of diatoms is the five-volume set, *The Freshwater Flora of Central Europe* (Krammer and Lange-Bertalot, 1986-1991, 2000).

More recent work from Lange-Bertalot (Diatoms of Europe, 2000-2013) adopts a finer taxonomic perspective. Bahls (2012) estimated that for the north and central portions of the western United States, only half of the taxa are documented in Krammer and Lange-Bertalot (1986-1991). Patrick and Reimer (1966, 1975) in *The Diatoms of the United States* brought a huge advantage over previous floristic works on diatoms. It is important to note that these references consider only a limited number of species, and exclude the centric and keel-forming taxa.

No one flora is currently available to address all the diatom species occurring in California, therefore, the taxonomic laboratory must combine resources (floristic and primary literature) to accurately identify the diatoms, keeping in mind that many new freshwater diatom species have recently been described to science from the western US (Kociolek et al., 2014) and elsewhere in the US (Morales, 2005, Morales et al., 2012, Morales and Manoylov, 2009, Potapova, 2012, Spaulding et al., 2010, etc.).

#### **1.4 Taxonomic Nomenclature**

When reporting algae results to SWAMP, all laboratories are required to use the same compilation of taxon names. To ensure data comparability, SWAMP maintains an Algae Master Taxa list. The list is accessible online at [http://swamp.waterboards.ca.gov/swamp\\_checker/LookUpLists.php](http://swamp.waterboards.ca.gov/swamp_checker/LookUpLists.php), and organized into two sections by the type of sample:

**SWAMP Master Taxa List-SBA** (OrganismLookUp - CAD-TWG Algae List)

**SWAMP Master Taxa List-Diatoms** (OrganismLookUp - CAD-TWG Diatom List)

The SWAMP Master Taxa List attempts to represent the most up-to-date, commonly accepted taxonomic scheme for each name. For each final ID, it includes taxonomic classification (phylum, class, order, family, genus, species, variety, form) and the taxonomic authors of the name.

When taxon names of SBA or diatoms are not available in the current SWAMP Algae Master Taxa List, the specimens must be well documented (see Section 1.5 and Appendix H) and submitted for taxonomic harmonization (see Section 1.7). Following approval, all final ID names for newly reported species must be reported in the Organism\_DetailLookUp

tab of the Taxonomy Results template (see Section 6 and Appendix G) using the following standard:

- Taxonomic classification should follow [Algaebase](#) (Guiry and Guiry, 2015);
- Species names with taxonomic authors should be obtained from the [Algaebase](#) website. Only use names that are currently accepted taxonomically (indicated by 'C');
- Taxonomic authors should be abbreviated according to the [International Plant Names Index](#). Since the SWAMP reporting format does not allow the use of periods in the name (in abbreviations, such as var., f., cf.), they cannot be included in the result. For example, *Cocconeis placentula* var. *lineata* (Ehrenberg) Grunow should be submitted as *Cocconeis placentula* var *lineata* (Ehrenb.) Grunow; and *Rhizoclonium* cf. *hieroglyphicum* (C. Agardh) Kützing should be submitted as *Rhizoclonium* cf *hieroglyphicum* (C. Agardh) Kütz.
- For taxa identified at genus or coarser taxonomic levels, a unique number in numerical order should be added to the name in agreement with existing names and numbers in the SWAMP Algae Master Taxa list. For example, *Calothrix* spp. should be submitted as *Calothrix* sp 9.

## 1.5 Photographic Documentation of Algae

Photomicrographs of algae provide an excellent source of documented information about the samples, and laboratories should include collection of photomicrographs in their standard procedures. Taking multiple photomicrographs of every species would generate a large amount of potentially useful supporting documentation.

In order to minimize the resources required, while maximizing the impact on data quality, SWAMP has identified two situations encountered during taxonomic analysis in regards to photographic documentation of algae (e. g. newly recorded algae taxa and previously reported taxa to SWAMP) The requirements and recommendations related to photographic documentation have been determined for each.

### 1.5.1 Photographic Documentation of Newly Reported Taxa

Newly reported taxa are those which have been previously described elsewhere or not, but have not been previously reported to the SWAMP Algae Master Taxa list. Some of these



newly reported species to the Algae Master Taxa list can be potentially newly discovered to science. SWAMP requires collection of high-quality photomicrographs for each newly reported taxon submitted to the Algae Master Taxa list. Newly reported taxa require the largest number of photomicrographs, as they provide critical information for the harmonization process.

While SWAMP requires taking photomicrographs of newly reported algae, a specific minimum number of photomicrographs is not established. The appropriate number of photomicrographs needed varies and should be determined by the taxonomist. The number of photomicrographs taken should be sufficient to support identification and harmonization of the new taxa.

#### **1.5.1.1 Photographic Documentation of Newly Reported SBA Taxa**

Photomicrographs must be taken of each newly reported SBA taxon identified. The number of photomicrographs taken should be sufficient to illustrate all diagnostic features and morphological variation needed for identification (see Appendix H). Some macroalgal taxa may require more than one photomicrograph at low and high magnification if several features are necessary for identification (e.g., key vegetative and reproductive characteristics). Microalgae may also require multiple photomicrographs of the characteristics of the colony, single cells from the colony, and specific diagnostic organelles. Images should be well focused on the key features. For the definition of macroalgae and microalgae see section 4.1.

#### **1.5.1.2 Photographic Documentation of Newly Reported Diatom Taxa**

For every newly reported diatom taxon that is identified, the slide on which it was seen and its position on the slide should be documented. Short descriptions with detailed observations about its frustular morphology as well as photomicrographs of the taxonomic entity should be provided. Depending upon the number of specimens and the variability expressed in the taxon, approximately five images per taxon showing the morphological variability should be collected (see Appendix H).

#### **1.5.2 Photographic Documentation of Previously Reported Taxa**

Previously reported taxa are those already included in the Algae Master Taxa list. SWAMP

strongly recommends that laboratories take representative photomicrographs of each SBA and diatom taxon identified in the sample, however, these do not warrant the same level of documentation required for newly reported species. Regardless, photomicrographs of reported species provide valuable information that supports the data quality at multiple levels (laboratory, project, and program).

SWAMP recommends laboratories take multiple photomicrographs of highly variable species from samples originating from distant locations. These photographs support consistency between identification of the algae taxa across the region.

### **1.5.3 General Requirements for Photographic Documentation**

All photomicrographs must be taken using TIFF format (without compression). Although TIFF files are significantly larger than files using alternate formats, they provide the high-resolution required, in addition to being the standard format required by many scientific publications.

Photomicrograph documentation of SBA or diatom specimens must have the following:

- Scale bar in the lower right corner of the image measuring 10, 20 or 50  $\mu\text{m}$  proportional to the size of the algae and magnification used;
- Be saved in TIFF format using the maximum resolution afforded by the equipment in use (minimum of 300 dpi);
- Each photomicrograph should have a filename consisting of the following elements in the order indicated: SWAMP Sample ID, Sampling date (MM/DD/YYYY), Species ID, magnification of the objective (i.e., 40x).

For example, the filename would be: 503ABC015\_Calothrix epiphytica\_07292014\_40x.tiff

The laboratory should store all photomicrographs on a high-capacity internal hard drive of the laboratory computer which is periodically backed up onto an external hard drive.

### **1.6 Standard Taxonomic Effort**

A standard taxonomic effort (STE) refers to the taxonomic level at which specimens must

be identified. Effort is required to achieve species level of algae identification, or the lowest taxonomic level possible. In the sections below, some limitations in achieving species level identification of SBA and diatoms are outlined. Laboratories are responsible for following the STE to ensure proper level of algae identification.

### **1.6.1 Standard Taxonomic Effort for SBA**

SBA species level identifications require observations of large portions of the filaments or colonies, and the presence of specific vegetative and reproductive structures. Absence of these structures can limit identification for some taxa to genus or coarser levels. The analysis of fresh algal qualitative samples and separate identification of the macroalgal fraction of quantitative samples applied in this SOP supplies additional morphological information facilitating species identification of problematic genera (Stancheva et al., 2012a). For instance, the species level identification of the following genera: *Anabaena*, *Dolichospermum*, *Cylindrospermum*, *Batrachospermum*, *Sirodotia*, *Oedogonium*, *Spirogyra*, *Zygnema*, *Mougeotia*, *Vaucheria*, etc. is largely based on their reproductive structures or specialized cells, such as akinetes. Non-reproducing specimens are more commonly observed. Therefore well-defined “morphospecies” are assigned for the non-reproductive specimens based on their vegetative morphology and are available in the California Online Algae Identification Resource Tools - [Soft-Bodied Stream Algae of California](#) (Stancheva et al. 2014). SWAMP requires that laboratories follow the taxonomic concept of accepted names presented in the California Online Algae Identification Resource Tools in order to facilitate consistent usage of SBA names.

Current taxonomic literature does not include all SBA taxa present in the US flora. The number of unknown and newly described species in the freshwater SBA flora of California is significant (Stancheva et al., 2012b, 2013, Hall et al., in preparation). During the analysis of samples, SBA taxonomists will record specimens that may appear either new to science or previously unreported in the SWAMP Algae Master Taxa list. Therefore, it is best to describe the unknown morphological entities well and to distinguish them from the established nomenclature.

### **1.6.2 Standard Taxonomic Effort for Diatoms**

Diatoms are typically identified to species level or lower because: (1) reproductive features are typically not required; (2) detailed and diagnostic features of the frustules can be seen

with good optics; and (3) frustules can be mounted on permanent slides with no loss of critical features facilitating detailed study and repeated observations over time among multiple specimens and researchers.

Every attempt should be made to make the identification of specimens to the finest level, however, there are situations where identification of specimens cannot be made to the level of species or finer due to its permanent position on the slide (for instance in girdle view). In these instances, the taxonomist should identify the specimen to the finest taxonomic level afforded such as genus, or occasionally family.

Current publications do not consider all taxa present in the US flora, therefore it is best not to “shoehorn” unknown morphological entities into established nomenclature. The number of unknown taxa in the freshwater diatom flora of California is significant (Kociolek et al., 2014). During the evaluation of samples, diatom taxonomists will undoubtedly encounter specimens that may appear either new to science or previously unreported in the SWAMP Algae Master Taxa list. It is always easier to combine names or designations with other species during harmonization than it is to try to tease out counts for two taxa that were originally reported under one name. A developing, critical mass of on-line guides such as [Diatoms of the United States](#) (Spaulding et al., 2010) is emerging and although not mature in a variety of ways (number of taxa presented, utility and ease of navigation of the sites), they are still very helpful and will become even more helpful in the future. The California Online Algae Identification Resource Tools - [Diatoms of the Southern California Bight](#) (Kociolek, 2012), provides a useful online reference for stream diatoms from southern California.

### **1.7 External Taxonomic Harmonization Process**

All newly reported taxa, some of which may be potentially newly discovered to science species, must undergo taxonomic harmonization before they are reported to SWAMP. The harmonization is a requirement for SWAMP datasets, and is recommended for non-SWAMP datasets. Harmonization is needed in order to load data into the SWAMP database (see appendix G for details). Taxonomic harmonization ensures that:

- The taxonomic nomenclature used to report SWAMP data is consistent with the Algae Master Taxa list;

- Identification of newly reported taxa is verified prior to reporting; and
- The Algae Master Taxa list is consistently updated to include newly reported taxa names.

An algal taxonomist from the California Primary Algae Laboratory (CalPAL) with extensive experience in SBA and diatom taxonomy of the local algal flora included in the SWAMP data set is authorized to lead the taxonomic harmonization process. The CalPAL taxonomist is also responsible for reviewing and approving new algal names produced by all laboratories performing algae analysis for SWAMP.

Harmonization is mandatory for newly reported taxa included in the dataset; however, it is not required for all previously reported species. Harmonization of the entire dataset would improve the overall quality of the reported results and has been identified as a future goal. SWAMP recommends harmonization of the entire dataset (including results from previously reported species), but does not currently require this step due to resource limitations.

The taxonomic harmonization process is identical for both SBA and diatoms. Harmonization requires communication between both taxonomists, achieved in part by the exchange of photographic documentation and text descriptions of SBA and diatoms. This process is time consuming for both taxonomists, therefore, efforts should be made from the primary taxonomist to reduce the number of new SBA and diatom names submitted for approval as follows:

- Each new species ID name must be checked for synonyms available in the Algae Master Taxa list.
- Each new genus level ID must be checked for comparability with all “morphospecies” in numerical order belonging to the same genus using the resources available online: [Soft-Bodied Stream Algae of California](#), and [Diatoms of the Southern California Bight](#).
- When observation and documentation of the morphological features of a new genus level ID are not possible due to the limitations outlined in Section 1.6, loose genus name categories should be used, such as *Achnathes*, *Navicula*, *Gomphonema*, *Anabaena*, etc. Genus identifications can be confirmed by consultation with the CalPAL taxonomist if needed.

All newly reported SBA and diatom names identified and verified as indicated above should be submitted to the CalPAL taxonomist along with high-quality photomicrographs of the determined taxon and a short morphological description, including the cell dimensions (see Section 1.5 and Appendix D for details). For distinct taxa identified to the genus or coarser taxonomic levels, the description should be focused on important morphological taxonomic features that make the taxon unique, including size measurements, allowing assignment of an unique number in numerical order. Some of these taxa may eventually be identified to species level when more information is accrued. It is critical that all documentation, characteristics, and descriptions are clear and provide enough detail to allow another taxonomist to understand the new diagnosis. The *Taxonomic Harmonization Datasheet* (Appendix D) is prepared by the primary taxonomist and submitted to the CalPAL taxonomist, who provides comments and recommendations, and approves the final taxa IDs after communication with the primary taxonomist. The review of some taxa ID may require checking the original sample from the CalPAL taxonomist. When the harmonization process is completed, all approved new algal names must be entered by the primary taxonomist in the Organism\_DetailLookUp tab of the Taxonomy Results template (see Section 1.4) and then all data can be reported to SWAMP (see Section 6 and Appendix H).

### 1.8 Training

Laboratories must have internal procedures for executing and documenting the training of laboratory technicians and taxonomists in the use of these procedures. Training is conducted by the experienced taxonomist. Documentation of training should include demonstration of performance in the procedures.

### 1.9 General Taxonomic Laboratory Practices

Good general laboratory practices include, but are not limited to, maintenance of the following:

- Written laboratory procedures clearly documenting all laboratory processes;
- Sample tracking and data management systems including, but not limited to, data sheets;
- Clean working conditions, including clean instrumentation and tools, such as forceps, scissors, and other tools that come into contact with sample matrices;

- Clean microscopes, including objective lenses, eyepieces and light sources as necessary, or as recommended by the manufacturer;
- Access to all relevant scientific literature;
- For all chemicals, current Material Safety Data Sheets (MSDS) for all chemicals in the laboratory. MSDS sheets should be available to all laboratory staff;
- Adherence to safety rules for glassware, hot plates, and chemicals such as oxidizers, toluene, naphrax, formalin, and glutaraldehyde (see Appendix B).

## Section 2: Laboratory Sample Receipt

Three separate stream algae samples are collected in the field and delivered to the lab as described by Ode et al. (2015).

**SBA qualitative sample:** Unpreserved sample consisting of a composite of all types of SBA macroalgae visible within the stream reach. This sample is collected in a 100 mL Whirl-Pak<sup>®</sup> bag and kept cool (4°C) and dark until it is received by the laboratory.

**SBA quantitative sample:** Sample preserved with 2% glutaraldehyde in 50 mL plastic centrifuge tube. If the samples arrive unpreserved, follow steps listed in Section 2.2.2.

**Diatom quantitative sample:** Sample preserved with formalin in 50 mL plastic centrifuge tube.

Upon delivery, the laboratory technician receives, inspects, and documents the incoming samples.

- A unique laboratory sample identification code (lab sample ID) for internal tracking purposes may be assigned to each sample.
- The condition of each sample upon receipt is assessed against the SWAMP required sample handling criterion (see Section 5.3).

## 2.1 Sample Receipt

Upon receipt, the laboratory must confirm that the sample labels match the chain of custody (COC) forms and all samples are accounted for. Sample site IDs should be written legibly on labels. Copies of the COCs must be retained as a record.

## 2.2 Sample Integrity Check

Following receipt, the laboratory must inspect each sample and confirm sample integrity has been maintained to the level indicated. Sample handling requirements and associated corrective actions are specified in Table 1 and Table 2 of Section 5.3.1.

### 2.2.1 SBA Qualitative Sample Integrity Check

- Confirm the sample is received in a 100 mL Whirl-Pak® bag.
- Confirm the sample is cool (4°C) upon receipt. Note if warm on the COC.
- Inspect the sample for evidence of freezing. Note evidence of freezing on the COC.
- Inspect the sample for evidence of leaking during shipping. Leaking can result in cross contamination of samples. Note evidence of leaking on the COC.
- Confirm the sample has been received within 2 weeks of collection.

If the qualitative SBA sample is received more than 2 weeks from collection, or if the integrity of the sample upon receipt is in question, the taxonomist must inspect the sample to determine the extent of sample degradation and document these findings on the COC.

### 2.2.2 SBA Quantitative Sample Integrity Check

- SBA quantitative sample may arrive unpreserved.
- Confirm that the SBA quantitative sample has been preserved. If the sample is received unpreserved, it must be preserved as soon as possible within 4 days of collection with 2% glutaraldehyde final concentration. The volume of the unpreserved sample, amount of glutaraldehyde added, and date and time of preservation must be documented on the COC.
- Samples preserved in the field are preserved with 2% glutaraldehyde in 50 mL plastic centrifuge tube.



- The total volume of the field-preserved sample should be 50 mL (45 mL sample and 5 mL preservative). Vials received with less than 50 mL of preserved sample may indicate the sample was not preserved or had leaked during transport.

### **2.2.3 Diatom Quantitative Sample Integrity Check**

- Confirm the samples are received preserved in the field with formalin in 50 mL plastic centrifuge tube.
- Inspect the volume in the sample vial. The total volume of the field-preserved sample should be 50 mL (40 mL sample and 10 mL preservative). Vials received with less than 50 mL of preserved sample may indicate the sample was not preserved or had leaked during transport.

### **2.2.4 Receipt of Broken Sample Vials**

If a vial is cracked or leaking it must be transferred to a new vial according to the following procedure:

- Transfer the affected sample to a new 50 mL plastic centrifuge tube with a label containing the sample information.
- Measure and record the remaining sample volume.
- Document the sample condition.
- Add additional preservative and note volume added on COC.

Note any action taken on the COC and notes section of the laboratory database sample log in.

## **Section 3: Sample Preparation**

After algal samples are received, samples are prepared for taxonomic analysis. The sample preparation process is different for the three different algae samples: (1) SBA qualitative sample; (2) SBA quantitative sample (macroalgae and microalgae fractions), and (3) Diatom quantitative sample.

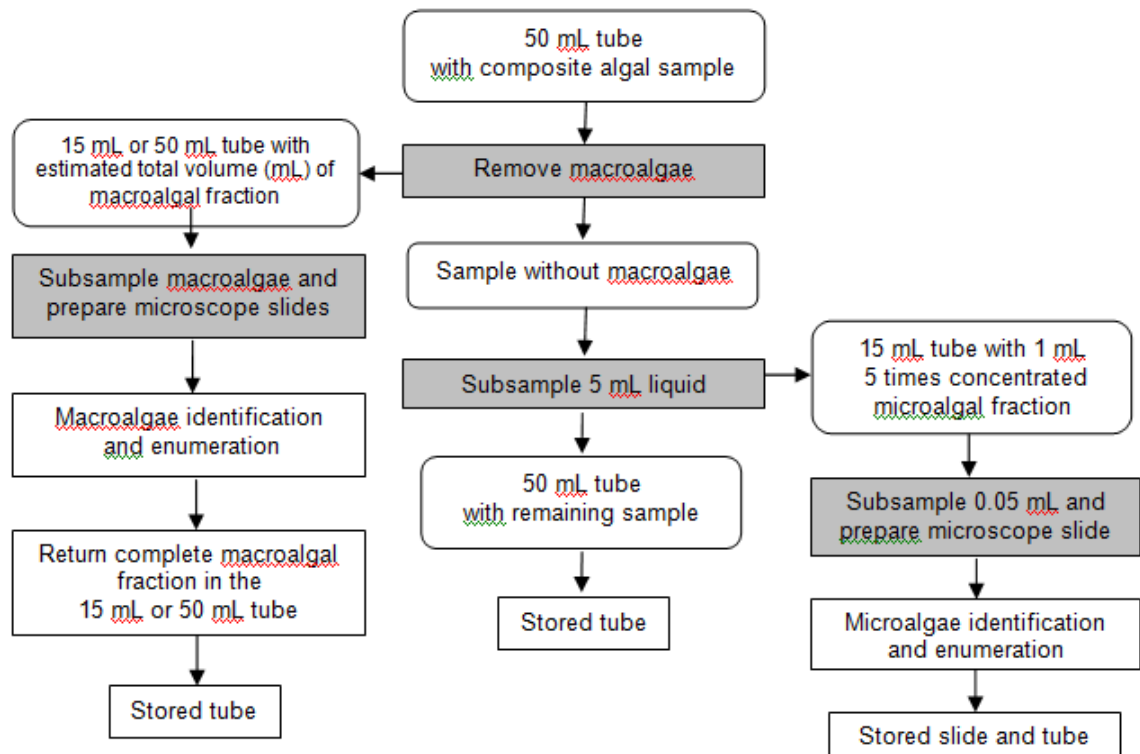
The purpose of analysis of qualitative SBA samples is to identify as many macroalgal taxa present in the sample as possible. Macroalgal species identification requires observation of enough unfixed material representing different life stages to determine vegetative features, reproductive mode, and characteristics of completely developed reproductive structures of each species. All macroalgal taxa are identified to lowest possible taxonomic level (usually to species).

Quantitative SBA samples contain algae of different sizes requiring detailed observations of many cellular, vegetative and reproductive structures in order for the species to be identified. For proper identification and enumeration of SBA taxa, macroalgal and microalgal fractions of each sample are processed separately (Figure 1).

The purpose of analysis of quantitative SBA samples is to identify as many SBA taxa present in the sample as possible, to provide an accurate algal taxa list and uniform biovolume estimate of each algal taxon in a sampled stream reach. This procedure is designed to produce a comprehensive list of all algal taxa identified to lowest possible taxonomic level (usually species) together with a precise estimate of their individual volumetric contribution per unit area sampled.

The purpose of the quantitative analysis of diatoms is to identify 600 valves of diatoms to the lowest possible taxonomic level (usually species) and to determine the relative abundance of the diatom taxa. For proper identification of diatoms, the diatom frustules need to be cleaned by removing all organic contents of the diatom cells.

During the sample preparation and consequent taxonomic analysis, care should be given to avoid sample cross contamination by using disposable materials, or carefully washed and DI rinsed materials. Instrumentation should be used only for an individual sample and then immediately stored for decontamination. Dropper bottles with DI or Lugol's Iodine Solution, used multiple times, should not touch the algal material. Sample splashing should be avoided when multiple samples are processed.



**Figure 1. SBA quantitative sample preparation flowchart.**

### 3.1 SBA Qualitative and Quantitative Sample Preparation

This section describes initial preparation of SBA samples for taxonomic analysis.

The processed samples are used for semi-permanent water mounts prepared by the taxonomist prior to algae identification and enumeration (Sections 3.1.4 and 4.1).

#### 3.1.1 SBA Qualitative Sample Preparation

##### Materials needed:

- Fresh sample in a 100 mL Whirl-Pak® bag
- Glass specimen dish
- Forceps (30 cm long) and jewelers forceps
- DI water
- Beakers (50 mL)
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Dissecting and compound microscope, each with digital camera

**Step 1:** Very gently transfer the fresh macroalgae from the field plastic bag into a glass dish containing DI water.

**Step 2:** When the taxonomic work on the sample is completed (see Section 4.1.1) archive a portion of the fresh sample (see Section 4.3.1). Archiving of the SBA qualitative sample should be conducted as soon as possible following completion of the taxonomic analysis. Return the remaining material to the original plastic bag, loosely capped, adding DI water if needed. The fresh sample should be archived for two more weeks in the refrigerator at 4°C in case further examination is needed.

### 3.1.2 SBA Quantitative Sample Preparation – Macroalgal Fraction

#### Materials needed:

- Preserved composite sample in 50 mL plastic centrifuge tube
- Forceps (30 cm long) and jewelers forceps
- DI water
- 15 mL graduated centrifuge tube with graduations in 0.1 mL increments up to 1 mL, and 0.5 mL increments above
- 50 mL graduated centrifuge tube with graduations in 2.5 mL increments
- Grid bottom culture dish
- Microscope slides
- Cover slips - 22 x 30 mm, No 1 thickness
- Dissecting and compound microscope, each with digital camera

**Step 1:** Obtain the 50 mL centrifuge tube with preserved composite sample and visually inspect its content to estimate whether a 15 mL or 50 mL tube is needed for macroalgae fraction collection.

**Step 2:** Label a 15 mL or 50 mL graduated centrifuge tube with the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)
- Note “macroalgae” on the label to distinguish from the microalgae fraction
- Macroalgae volume: xx mL

If the macroalgal fraction is very large, use a 50 mL graduated centrifuge tube with 2.5 mL increments.

**Step 3:** Place 10 mL of DI water into the labeled centrifuge tube.

**Step 4:** Using the forceps (30 cm long), very gently pinch the material at the bottom of the tube. Search for visible macroalgal clumps, and any solid particles in the sample, such as mosses, vascular plant tissues, roots, etc. Gently pull up the forceps and slowly move the macroalgae and all solid particles grasped between the forceps in the solution to remove extra clinging sediment and isolate any macroalgal filaments in the sample. Repeat this step at least three times before proceeding to the next step.

**Step 5:** If macroalgal clumps are present in the sample continue onto Step 6. If no macroalgal clumps are present, proceed with preparation of the microalgae fraction (Section 3.1.3). If no macroalgae and any solid particles are visible to the naked eye, inspect the sample tube under a dissecting microscope before proceeding with microalgae preparation.

**Step 6:** Using forceps, remove the macroalgae from sample very gently, squeeze it to remove as much liquid as possible and then place it into the tube with 10 mL DI water. Continue until no macroalgae remain.

**Step 7:** Determine the volume of macroalgal fraction by the increase (displacement) from the original 10 mL of water. When using 15 mL centrifuge tubes with graduated markings measuring 0.5 mL, estimate the water displacement to 0.1 mL (See Note 1 below). Record the volume of the macroalgal fraction (mL) in the *ID Datasheet for SBA Sample- Heading: Qualitative sample – Heading: Macroalgal fraction-total volume: xx mL* (Appendix C1) and on the label of the tube with the macroalgal fraction.

**Note 1:** The surface of water in a tube is not completely flat. Instead, the surface curves in a shallow U-shape meniscus. When measuring, read the line just at the bottom of the meniscus.

### 3.1.3 SBA Quantitative Sample Preparation – Microalgal Fraction

**Materials needed:**

- Preserved composite sample in 50 mL plastic centrifuge tube remaining after macroalgae removal
- 10 mL pipette
- 50 mL centrifuge tube with graduations in 2.5 mL increments
- 15 mL centrifuge tube with graduations in 0.1 mL increments up to 1 mL, and 0.5 mL increments above
- Dissecting needles
- Table-top centrifuge
- 146 mm borosilicate pipette
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Compound microscope with digital camera

**Step 1:** Obtain the 50 mL centrifuge tube containing the SBA quantitative sample following removal of the macroalgae fraction. Homogenize the microalgal fraction of the SBA quantitative sample by gently but thoroughly inverting the centrifuge tube several times. The sample must be well homogenized prior to sub-sampling (Step 2).

**Step 2:** Pipette 5 mL of homogenized microalgae fraction into a 50 mL centrifuge tube labeled with the sample information.

**Step 3:** Fill the centrifuge tube with DI water to the 50 mL mark. Let the sample settle for a minimum of 12 hours.

**Step 4:** Once the sample has thoroughly settled, gently remove the supernatant layer down to a volume of 5 mL by using a pipette. Avoid disturbing the algal material on the bottom of the tube.

**Step 5:** Label a 15 mL graduated centrifuge tube with the following information:

- SWAMP sample ID

- Date of collection (MM/DD/YYYY)
- Note “microalgae” on the label to distinguish from the macroalgae fraction

**Step 6:** Transfer the 5 mL of sample material from the 50 mL centrifuge tube to the labeled 15 mL graduated centrifuge tube.

**Step 7:** Rinse down the sides of the 50 mL centrifuge tube several times with DI water to capture any remaining algae clinging to the sides. Transfer the rinse liquid to the labeled 15 mL graduated centrifuge tube.

**Step 8:** Fill the labeled 15 mL graduated centrifuge tube with DI water to the 15 mL mark.

**Step 9:** Centrifuge the sample for 5 min at 4000 RPM on a table-top centrifuge.

**Step 10:** Remove the supernatant layer until 1 mL sample is left by using a pipette. Avoid disturbing the algal material on the bottom of the tube.

This procedure concentrates the microalgal fraction 5 times while removing most of the glutaraldehyde before microscopic examination. From this 1 mL sample, a semi-permanent slide is prepared for analysis (Section 3.1.4 below).

### **3.1.4 SBA Semi-permanent Slide Preparation of Quantitative Microalgal Fraction**

#### **Materials needed:**

- 15 mL centrifuge tube with microalgal fraction of 1 mL
- DI water
- Dissecting needles
- 146 mm borosilicate pipette
- Microscope slides
- Cover slip - 22 x 30 mm, No 1 thickness
- Nail polish
- Compound microscope with digital camera

**Step 1:** Obtain the 15 mL centrifuge tube containing 1 mL concentrated microalgal fraction.

**Step 2:** Visually inspect the sample and evaluate the amount of material (sediment and

algae) settled on the bottom of the tube. If the sample contains more than 0.5 mL of material (sediment and algae) settled on the bottom, dilute with DI water to a final volume of 2, 3, 4 or 5 mL.

If a small amount of material (sediment and algae) is present, centrifuge for 5 min at 4000 RPM on a table-top centrifuge and concentrate the sample to 0.5 mL.

Record any additional dilution or concentration performed on the sample and the final sample volume used for slide preparation in the *ID Datasheet for SBA Sample-Heading: Quantitative sample-Microalgal fraction-Sample volume after additional dilution/concentration: xx mL* (Appendix C1). This information is required for SBA biovolume calculations (see Section 4.1.4.2, Appendix F).

**Step 3:** Vortex or pipet-mix the sample and subsample with pipette from the center of the well-mixed material. Place 1 drop (0.05 mL) of sample on a standard microscope slide and cover with a 22 x 30 mm cover slip.

Proper preparation of the slides is vital to performing identifications. The following should be noted while preparing slides:

- Ensure that the volume of the drop is not so large that it creates the formation of bubbles or causes the cover slip to float.
- Avoid having too much or too little material on the slide. Too much material results in layers of cells, specimen overlap and a non-flat cover slip which interferes with accurate identification. Too little material increases the amount of time required to complete analysis and may not be adequate for proper identification and enumeration.
- Small thick clumps of spreading filaments intermixed with colonial algae can sometimes occur in the microalgal fraction. Clumping of material not only interferes with accurate identification and enumeration, but can circumvent the assumption of random distribution of specimens on the slides. These clumps usually contain several different species, so they should to be dispersed before proceeding with analysis. Gentle tapping on the cover slip or spreading the clump apart with a pair of dissecting needles will reduce clumping.



**Step 4:** Inspect the semi-permanent microalgal slide at lower magnification (200x) using a compound microscope to confirm that microalgae are evenly distributed. Gently adjust the cover slip if algal clumps are present. Cover slip may be sealed with nail polish to prevent evaporation. This semi-permanent microalgal mount is good for analysis for at least two hours.

### **3.2 Diatom Quantitative Sample Preparation**

Two methods are available for diatom cleaning: the nitric acid method (American Public Health Association, 1981) and the hydrogen peroxide and potassium dichromate method (Van der Werff, 1955).

#### **3.2.1 Cleaning of Diatom Samples: Nitric Acid Method**

Concentrated nitric acid is extremely hazardous, and therefore specific handling and disposal procedures must be in place. Staff should consult the appropriate MSDS provided by the supplier. Nitric acid should always be handled in a positive-draw fume hood by trained staff wearing safety goggles, rubber gloves, and lab coats. Diatom quantitative samples are preserved in formalin, so they must be handled carefully.

#### **Materials needed:**

- Preserved diatom sample in 50 mL plastic centrifuge tube
- Beakers (250 mL)
- Concentrated nitric acid ( $\text{HNO}_3$ )
- HCE (10%)
- Hot plate
- Centrifuge
- 15 mL centrifuge tubes
- DI water
- pH paper
- Waste container
- Scissors

**Step 1:** Label each beaker with tape and the corresponding sample number.

**Step 2:** Obtain the preserved diatom sample in 50 mL plastic centrifuge tube. Check

sample to determine if macroalgal clumps are present. If large algal clumps are observed in the sample, cut them into smaller pieces with scissors.

**Step 3:** Shake the sample vial vigorously and pour 20 mL (30 mL for sparse samples) of homogenized sample into the beaker labeled with the sample information.

**Step 4:** Add a small amount of nitric acid to the beaker to test if a violent or exothermic reaction occurs. If violent reaction does occur, or if carbonates are abundant in the sample, the sample can be pre-treated by adding 10% HCl.

**Step 5:** When it has been determined that no violent reaction will occur, slowly add the remaining volume of nitric acid to the beaker. In all, a volume of nitric acid approximately equal to the volume of sample processed is added to the beaker.

**Step 6:** Place the beaker on a hot plate under a positive-draw fume hood. Boil the sample and nitric acid mixture until the organic content turns white. This white material is the siliceous cell walls of the diatoms. This step typically takes 30 minutes to 1 hour, during which the volume of the material will be reduced to about  $\frac{1}{2}$  (see Note 2 below).

**Step 7:** Once the boiling step is complete, allow the sample to cool. Transfer the sample to a 15 mL centrifuge tube labeled with the SWAMP sample ID and centrifuge at 3500 rpm for 8 minutes.

**Step 8:** Pour the supernatant off into an appropriately designated waste container. Add DI water to the centrifuge tube containing the diatom sample and centrifuge at 3500 rpm for 8 minutes.

**Step 9:** Repeat the cycle of decantation, addition of new DI water, and centrifugation 5 times, or until the pH of the water is neutral (or the same as the deionized water being used – may not be pH 7). The result should be a pellet of nearly white material at the bottom of the tube.

**Step 10:** Label a 15 mL graduated centrifuge tube with the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)
- Note “diatoms” on the label to distinguish from the other samples

**Step 11:** Transfer the cleaned diatom material to the labeled 15 mL centrifuge tube. Add DI water up to the 10 mL mark. This is the material from which the slides will be made.

**Note 2:** The surfaces of hot plates can get hot enough to cause boiling over or explosive conditions, especially with samples containing high amounts of organics or carbonates. Alternative methods such as using a heating block with 26 mm diameter glass test tubes (the lower portion of each tube stay hot while the upper portion stays cool, creating a reflux action, minimizing the risk of over-boiling or drying the sample) or microwave apparatus (Acker et al., 2002) may be used to clean the diatoms.

### 3.2.2 Cleaning of Diatom Samples: Hydrogen Peroxide Method

The principal reagents used in this method, hydrogen peroxide and potassium dichromate, are extremely hazardous. 30% hydrogen peroxide is a strong oxidizer, and may require special handling and storage. Specific handling and disposal procedures must be in place for the handling these materials. Staff should consult the appropriate MSDS provided by the supplier. Both chemicals must always be handled in a positive-draw fume hood, and staff should wear safety goggles, nitrile gloves and lab coats.

#### Materials needed:

- Preserved diatom sample in 50 mL plastic centrifuge tube
- Beakers (250 mL)
- Hydrogen peroxide ( $\text{H}_2\text{O}_2$  30%)
- Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ )
- Microspatula
- Squirt bottle of DI water
- DI water
- Centrifuge
- 15 mL centrifuge tubes
- Waste container
- Scissors

**Step 1:** Label each beaker with tape and the corresponding sample number.

**Step 2:** Obtain the preserved diatom sample in 50 mL plastic centrifuge tube. Check the

sample to determine if macroalgal clumps are present. If large algal clumps are observed in the sample, cut them into smaller pieces with scissors.

**Step 3:** Shake the sample vial vigorously and pour 20 mL (30 mL for sparse samples) of homogenized sample into the beaker labeled with the sample information.

**Step 4:** Add approximately 20-30 mL of 30% hydrogen peroxide to the sample.

**Step 5:** Place the beaker on a hot plate under a positive-draw fume hood. Bring to boiling.

**Step 6:** Remove from heat and immediately add a small amount (several crystals) of potassium dichromate to the mixture using a microspatula. The addition of the potassium dichromate will catalyze a strong exothermic reaction, therefore, the potassium dichromate should be added slowly. Have a squirt bottle of DI water at the ready in case the reaction begins to boil over the top of the beaker. The reaction takes approximately 5 to 10 minutes to complete. Completion of the reaction is indicated by solution changing in color from dark purple to orange.

**Step 7:** Add DI water up to 200 mL.

**Step 8:** Allow the diatom material to settle for at least 8 hours.

**Step 9:** Slowly and gently, to avoid disturbing the diatom material on the bottom of the beaker, decant the liquid into an appropriately designated waste container.

**Step 10:** Refill beaker with the diatom material on the bottom with DI water to 200 mL.

**Step 11:** Repeat steps 8-10 several times (approximately 3 to 5) until the cleaned diatom material is mostly colorless.

**Step 12:** Let the cleaned diatom material settle overnight and decant the supernatant as low as possible.

**Step 13:** Label a 15 mL graduated centrifuge tube with the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)
- Note "diatoms" on the label to distinguish from the other samples

**Step 14:** Transfer the cleaned diatom material to the labeled 15 mL centrifuge tube. Add DI water up to the 10 mL mark. This is the material from which the slides will be made.

**Note 3:** The method of allowing the material to settle over 8 hours can also be applied to the nitric acid procedure; however, since the resulting nitric acid solution is often colorless, testing the pH of the solution is a more reliable way to determine when the material has been sufficiently rinsed. Centrifugation of the sample to remove the remaining chemicals from hydrogen peroxide procedure can also be used.

### 3.2.3 Permanent Slide Preparation of Diatom Samples

Permanent slide preparation is the same, regardless of which cleaning method is chosen.

#### Materials needed:

- 15 mL centrifuge tube containing the cleaned diatom material
- Cover slips – 18 x 18 mm or 22 x 22 mm, No 1 thickness
- Microscope slide
- 146 mm borosilicate pipette
- Hot plate
- Naphrax
- Forceps
- Razor blade
- 70% Ethanol
- 10% HCL
- DI water

**Step 1:** Drip an amount of DI water onto the cover slip with a glass pipette. The amount should be sufficient to form a thin layer of water over the entire cover slip when the diatom suspension is added. If the clean diatom suspension is very sparse, skip this step.

**Step 2:** Obtain the 15 mL centrifuge tube with cleaned diatom material. Agitate the vial containing the cleaned diatom suspension and quickly withdraw material from near the central portion of the sample using the glass pipette.

**Step 3:** Eject one or two drops of diatom suspension smoothly and carefully into the layer

of DI water on the cover slip (see Note 4). If the clean diatom suspension is very sparse eject three to five drops directly onto a cover slip without DI water. If the cover slip overflows, discard it, clean the area, and prepare a new cover slip with diatoms.

**Step 4:** Air dry the material or gently dry the material on a warm hot plate. The temperature of the hot plate must not exceed 40°C. Higher temperatures cause the water to circulate or bubble, resulting in a non-random distribution or loss of diatom valves. Avoid any procedure that rapidly evaporates the suspension. Rapid evaporation could produce strong patterns of diatoms settling on the cover slip.

**Step 5:** When the cover slips have visibly dried, place them on a hot plate at an elevated temperature to drive off any remaining moisture.

**Step 6:** Confirm the prepared cover slip contains a random distribution of diatoms sufficiently dense for conducting identification and enumeration procedures. On average, 15 to 30 diatom valves should be visible in a single field of view. Confirm density and random distribution in 5 fields of view. If clumps of diatom valves are on the slide to the point where individual specimens cannot be viewed prepare another cover slip.

**Step 7:** Add a small amount of mounting medium (Naphrax) to a cleaned microscope slide and put the cover slip (diatoms down) on the mounting medium with forceps.

**Step 8:** Put the microscope slide with the cover slip on a hot plate preheated to 120 to 150°C. Leave on hot plate until bubbles stop forming under the cover slip, indicating that all the solvent from the mounting material has been driven out of the medium.

**Step 9:** Use forceps to safely remove the slide from the hot plate. Gently tap down on the cover slip to remove any air bubbles and to even the distribution of diatoms.

**Step 10:** Once the slide cools, scrape any excess mounting medium that remains outside the cover slip with a single-edged razor.

**Step 11:** Attach adhesive labels to the completed slides and include the following information:

- SWAMP sample ID
- Date of collection (MM/DD/YYYY)

- Note “diatoms” on the label to distinguish from the other slides

**Step 12:** Preserve the unused cleaned diatom material with ethanol to 50% final concentration and archive the vial (see Section 4.3.3).

**Note 4:** To achieve a more even distribution of diatom valves on the coverslip, 10% HCL can be added to the cleaned diatom suspension (1 drop per 10 mL of material in the vial).

## Section 4: Identification and Enumeration Analysis of Algae

### 4.1 Identification and Enumeration Analysis of SBA

Correct identification of benthic SBA taxa requires separation of the algae in the sample based on size classes into a macroalgal fraction and a microalgal fraction defined as follows:

**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 (e.g., *Nostoc*, *Rivularia*, *Batrachospermum*, *Lemanea*, *Cladophora*, *Draparnaldia*, *Oedogonium*, *Rhizoclonium*, *Spirogyra*, *Zygnema*, *Mougeotia*, *Vaucheria*) [see Sheath and Cole (1992) for definitions of forms].

**Microalgae** are small, microscopic forms not recognizable with the naked eyes but consisting of unicellular, colonial or filamentous non-diatom algae.

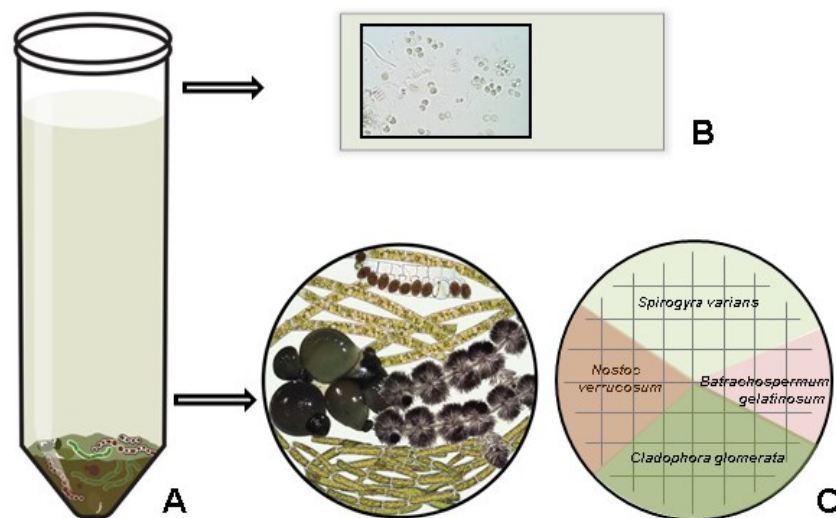
Proper identification of SBA requires a different approach for each fraction. Macroalgal species identification needs observation of enough material to adequately characterize vegetative and reproductive structures under a combination of dissecting and compound microscopes. Examination of microalgae should reveal cellular details, such as chloroplasts, pyrenoids, cell wall, among other features. Detailed and careful observations are necessary for accurate identification. Some diagnostic features are not evident without specific techniques, such as staining (e. g. starch with Lugol’s iodine solution). Use of taxonomic resources (see Section 1.3 and Appendix I) and taking photomicrographs while making identifications will facilitate taxonomic consistency and qualitative assurance.

Enumeration of the microscopic algae observed in the microalgal fraction, and as epiphytes in macroalgal fraction is based on a counting unit called a **natural counting entity (NCE)**.

**Natural counting entity (NCE)** is each natural occurring form of algae (i.e., each unicell, colony, filament, tissue-like form, coenocyte, tuft, or crust) regardless of the number of cells in the thallus or colony.

The main purpose of using “natural counting entity” is to prevent numerous small cells in a sample with macroscopic forms from dominating a count relative to their actual contribution to the community biomass. It also facilitates the counting of algal forms which have linked cells that may be hard to distinguish.

Laboratory set up for separate analysis of macroalgal and microalgal fractions of the SBA quantitative sample is illustrated in Figure 2 and explained in the Sections 3.1 and 4.1.



**Figure 2. Laboratory set up for separate analysis of macroalgal and microalgal fractions of SBA quantitative sample.**

**Legend:** **A.** 50 mL centrifuge tube containing SBA composite quantitative sample; **B.** Microalgal fraction placed on microscope slide with 22x30 mm cover slip. Typically 0.25 mL of the composite sample liquid is analyzed. **C.** Macroalgal fraction with measured total volume (i. e. 0.5 mL) placed in a grid Petri dish to estimate the proportions of species. In this case: *Cladophora glomerata* (31%), *Nostoc verrucosum* (24%), *Batrachospermum gelatinosum* (15%), and *Spirogyra varians* (30%).



#### 4.1.1 SBA Qualitative Sample Analysis

**Step 1:** Using a research-grade dissecting microscope carefully examine the macroalgal fresh sample placed into glass dish and determine the number of macroalgal genera in the sample appearing different in morphology or color.

Thoroughly examine all the material and identify key macroalgal features needed to separate the algae by genus. These may include:

- Colonial shape, size and color in cyanobacteria (such as *Nostoc*, *Dichothrix*, *Rivularia*);
- Different life stages, heterocyst position and akinete development in cyanobacteria (such as *Anabaena*, *Cylindrospermum*, *Gloeotrichia*);
- Male and female specimens with developed reproductive structures in red and green algae (such as *Batrachospermum*, *Sirodotia*, *Oedogonium*);
- Different life stages and completely matured reproductive structures in zygnematalean algae and tribophytes (such as *Spirogyra*, *Zygnema*, *Mougeotia*, *Vaucheria*).

**Step 2:** Place each macroalgal genus identified aside. When small rocks are collected, carefully examine the surface of the rocks for attached algae using dissecting microscope. If algae are present, scrape them out and place them on a microscope slide.

**Step 3:** Prepare microscope slides for each macroalgal genus which may be presented with more than one species in the sample. The number of slides prepared depends on the need to obtain sufficient information to successfully perform species identification.

When reproducing filaments of zygnematalean algae are observed, but completely matured zygospores/aplanospores are not available, further incubation under nutrient stress facilitates completion of sexual or asexual reproduction. The resulting mature zygospores (or akinetes, aplanospores) can provide the taxonomist with the additional information needed to identify the species.

To incubate the algae under nutrient stress:

- Select the conjugating filaments of *Spirogyra*, *Zygnema* or *Mougeotia* and place them in a 50 mL glass beaker filled with DI water.
- Keep the samples out of direct sunlight (in a north-facing window) at room temperature

until reproductive structures completely develop. Check for reproductive filaments under the microscope every three days and document the different stages of conjugation and development of zygospores (or akinetes, aplanospores).

**Step 4:** Examine prepared slides under the compound microscope and identify SBA macroalgae to species level. If large colonial diatoms are observed in the sample, record them following the recommendations in Section 4.1.2 Step 5.

**Step 5:** Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

**Step 6:** Record all macroalgal taxa identified in the SBA qualitative sample in the *ID Datasheet for SBA Sample* under the heading *Qualitative sample – list of taxa* (Appendix C1).

**Step 7:** Submit the remaining SBA qualitative sample and the algal material from the slides for archiving (see Section 4.3.1).

#### 4.1.2 SBA Quantitative Sample Analysis – Macroalgal Fraction

Macroalgal fractions extracted from different quantitative SBA samples vary considerably in total volume and content. Typically this fraction consists of soft-bodied macroalgae sometimes mixed with large colonial diatoms. However, due to the field sampling protocol (Ode et al., 2015), the macroalgal fraction may contain non-algal matter, such as mosses, vascular plant tissues, roots or debris, macroinvertebrates, etc., which should be subtracted from the macroalgal sample total volume. Many SBA and diatom macroalgae, as well as vascular plants, support development of epiphytic SBA algae, which are identified and enumerated also.

The approach for species identification of the macroalgae from the quantitative and qualitative samples (Section 4.1.1) is similar except for the process of algae incubation. Microscope slides for macroalgae and SBA epiphytic species identification need to be prepared during the identification process following the observations under the dissecting microscope.

**Step 1:** Start with the 15 mL or 50 mL centrifuge tube containing the macroalgal fraction extracted from the SBA quantitative sample.

**Step 2:** Transfer the contents of the centrifuge tube to a gridded Petri dish.

**Step 3:** Using forceps gently spread the material in the sample evenly throughout the Petri dish.

**Step 4:** Carefully examine the material under the dissecting microscope. If the sample contains non-algal matter such as mosses, vascular plant tissues, roots or debris, separate this material from the macroalgae and determine its volume. If it is possible to remove the non-algal matter, place it in centrifuge tube and measure the volume using water displacement. If the non-algal matter is not possible to remove, visually estimate its proportion. Calculate the fraction of the total sample volume represented by non-algal matter. Record the fraction represented by non-algal matter in the *ID Datasheet for SBA Sample-Heading: non-algal matter xx %* (Appendix C1).

**Step 5:** After the non-algal matter has been separated, gently distribute the soft-bodied macroalgae evenly using forceps. When working with the macroalgal fraction, care should be given to avoid breaking up large filaments or colonies and to preserve key features needed for species identification. Since large reproductive structures are easily damaged, the clumps should be spread very carefully.

If the sample contains macroalgal colonial diatoms (such as *Melosira*, *Pleurosira*, *Terpsinoe*, *Cymbella*, *Gomphonema*, *Didymosphenia*, *Bacillaria*, etc.) separate this material from the soft-bodied macroalgae and determine the representative proportion of the diatoms as a percent of total macroalgal fraction volume. If possible, identify diatoms to genus level, if not – use the general category “diatoms”. This information can help recording invasive or bloom-forming macroalgal diatom taxa, which may be omitted in diatom analysis due to numerical dominance of smaller diatom species.

**Step 6:** Using a dissecting microscope, thoroughly examine the sample and identify key macroalgal features needed to segregate the SBA by genus. Determine the number of distinct soft-bodied macroalgal genera present in the sample.

**Step 7:** Prepare microscope slides for each soft-bodied macroalgal genus. The number of slides depends on the need to obtain sufficient information to successfully perform identification and support the STE.

**Step 8:** Examine prepared slides under the compound microscope. Identify soft-bodied macroalgae to species level.

**Step 9:** Estimate the representative proportion of each soft-bodied macroalgal species as a percent of total macroalgal fraction volume in order to determine their biovolume (see Section 4.1.4.1).

**Step 10:** Record the name and the representative proportion of each soft-bodied macroalgal taxon identified in the *ID Datasheet for SBA Sample-Heading: Macroalgae taxon ID; Proportion of each taxon (%)* (Appendix C1).

**Step 11:** When the total volume of macroalgal fraction is so low that it is not possible to be measured by water displacement, prepare a microscope slide containing all macroalgal material. Under compound microscope identify all macroalgal taxa and determine directly their biovolume by individual measuring of each specimen using geometric shapes (such as cylinder for filamentous algae, or sphere for young colonies of *Nostoc*, for details see Section 4.1.3 Step 4). Calculate the total biovolume of each macroalgal species by summing the biovolumes of all specimens measured. In this case the biovolume of each macroalgal species is directly measured in  $\mu\text{m}^3$ .

**Step 12:** Identify to species level and enumerate 100 NCEs of epiphytic SBA attached to the surface of the soft-bodied macroalgae and other aquatic substrates. If fewer than 100 NCEs of epiphytic SBA are observed, enumerate as many as there are in the entire macroalgal fraction.

**Step 13:** Record each SBA epiphytic taxon identified and the corresponding number of NCEs enumerated in the *ID Datasheet for SBA Sample-Heading: Epiphyte taxon ID; #NCE* (Appendix C1).

**Step 14:** Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to

demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 Photographic Documentation of Algae and Appendix H).

**Step 15:** Return the entire content of the macroalgal fraction, including the material from the slides, and the non-algal matter into the tube with macroalgal fraction of the SBA quantitative sample and submit it for archiving (see Section 4.3.1).

#### 4.1.3 SBA Quantitative Sample Analysis – Microalgal Fraction

The microalgal SBA fraction is examined on a semi-permanent water mount for best observation of algal cellular morphology. Water mounts allow adjusting of the cover slip to change the position of the cells and spreading out of multilayered cell clumps for observation of critical taxonomic features. Furthermore, specific techniques, such as staining (e. g. starch with Lugol's iodine solution) are possible to apply on water mount.

**Step 1:** Using a research quality compound microscope, scan the semi-permanent slide with microalgae at magnification 200x to assess the taxonomic composition of the sample.

**Step 2:** Switch to a magnification of 400x (e.g., 40x objective with 10x eyepieces). At a magnification of 400x, the cover slip is composed of many horizontal optical transects.

**Step 3:** Identify and enumerate 300 SBA NCEs across a known number of horizontal optical transects. Count only intact cells with complete cell contents. If 300 SBA NCEs have been counted before reaching the end of the last transect, continue enumeration and complete the transect. This ensures that a known fraction of the sample is analyzed. Record the number of horizontal transects traversed, each SBA microalgal taxon identified and the corresponding number of NCEs enumerated in *ID Datasheet for SBA Sample-Heading: Microalgal fraction-number of transects counted: xx, Microalgae taxon ID; #NCE* (Appendix C1).

The taxonomist should adjust the total of NCEs enumerated under the following circumstances:

- If the sample contains numerous small single cells or new taxa appeared after counting 300 SBA NCEs, continue enumeration to 400 or 500 NCEs.

- If the morphological information obtained during the enumeration of 300 NCEs is not sufficient for species identification of certain taxon, continue observations of more specimens outside the counted area until species level identification is achieved.
- Stop counting after enumeration of the first complete slide if fewer than 20 SBA NCEs are recorded. Enumeration is stopped regardless of whether the sample received additional dilution or concentration prior to slide preparation.
- Prepare additional slides if more than 20 SBA NCEs are recorded in the first slide. Continue enumeration for 4 hours or until 150 SBA NCEs have been enumerated (whichever comes first). Exclude the time spent identifying and documenting new species from the total enumeration time.

**Step 4:** Using the techniques described by Hillebrand et al. (1999), determine the appropriate geometric model for each microalgal species identified. The list of geometric shapes corresponding to the SBA genera reported for SWAMP are detailed in Appendix E. Perform microscopic measurements of the cell dimensions for each algal entity according to the closest geometric shape. Record measurements in the *ID Datasheet for SBA Sample-Heading: Cell diameter ( $\mu\text{m}$ ); Cell/Filament length ( $\mu\text{m}$ ); Cell depth ( $\mu\text{m}$ ); Total number of cells; Total filament length ( $\mu\text{m}$ )* (Appendix C1).

The following should be noted while estimating biovolumes:

- Measurements for each single SBA NCE are made concurrent with identification and enumeration. Most microalgal NCE can be viewed as cylinders, spheres, spheroids, or cones in order to estimate biovolume. Proper biovolume estimate requires measurements of two or three dimensions, such as cell width, length and depth. Depth measurements are made on specimens from a side view. This can be achieved by lightly tapping the coverslip to turn the specimen. If the specimen cannot be measured from the side, estimate the depth and include a note in the data record. SBA size measurements should be made using an ocular micrometer or by image analysis software when photomicrographs are available.
- A multicellular coccoid colony is considered to be a single NCE. However, the number of cells in the colony varies among the species and in many taxa increases with the age

(for instance, cyanobacterial species belonging to *Aphanocapsa* and *Aphanothece*).

When the colony consists of a small number of cells, measure the dimensions of all individual cells. If the colony contains a large number of cells average the cell dimensions (diameter and length) obtained by measurement of 20 cells and record the total cell number.

- Often the colonies break into fragments with different sizes, each one of which is considered a NCE. The biovolume of colonial NCE is calculated by multiplying averaged cell biovolume by number of cells per colony.
- For a filament, which is considered a single NCE, measure the width and the whole length of the filament. When some filamentous taxa are abundant in the sample (such as, species belonging to *Leptolyngbya* and *Heteroleibleinia*) average the width and the length of the filaments based on the measurements of 20 NCE.
- However, size averaging should be applied only for an individual sample, and not extrapolated to other samples, because filaments and colonies break apart into fragments with different lengths under variable conditions.

**Step 5:** Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

**Step 6:** Submit remaining microalgal fraction of the SBA quantitative sample for archiving (see Section 4.3.2).

#### 4.1.4 Biovolume Calculations for SBA

The biovolume for each identified SBA taxon is measured during the identification and enumeration. Precision of the SBA biovolume estimates is achieved by separate processing of macroalgal and microalgal fractions and by cell size measurements made for each single microalgal NCE (see Stancheva et al., 2012a for details).

#### 4.1.4.1 Biovolume Calculations: SBA Quantitative Sample – Macroalgal Fraction

**Step 1:** Determine the representative proportion of each macroalgal species as a percentage of the total volume of macroalgal fraction, from which the non-algal matter has been subtracted, to calculate the biovolume of each species.

Determine the biovolume of each species in mL and convert it to  $\mu\text{m}^3$  when multiplied by  $10^{12}$ . Note that in some cases the biovolume of macroalgal species is directly estimated in  $\mu\text{m}^3$  (see Section 4.1.2 Step 11).

This is a total biovolume of i-species ( $V_a$ ) in macroalgal fraction of sample in  $\mu\text{m}^3$ .

**Step 2:** The value ( $V_a$ ) is multiplied by 4 in order to estimate the biovolume of each macroalgal species in the original field composite sample ( $V_a'$ ), from which only 1/4 of the macroalgae were transferred into the 50 mL tube received in the laboratory for SBA analysis (Ode et al., 2015, p. F-1).

$$V_a' = 4 V_a (\mu\text{m}^3)$$

Use the following formula to calculate the biovolume of each macroalgal species in  $\mu\text{m}^3$  per  $\text{cm}^2$  stream bottom area sampled. The result unit is  $\mu\text{m}^3\text{cm}^{-2}$ .

$$V_i = V_a' A^{-1} (\mu\text{m}^3\text{cm}^{-2})$$

where:

$V_i$  = biovolume of i-species ( $\mu\text{m}^3$ ) per 1  $\text{cm}^2$  stream bottom area sampled

$V_a'$  = biovolume of i-species ( $\mu\text{m}^3$ ) in the original composite sample

$A$  = stream bottom area of substratum sampled. It is the total area of substratum surface from which benthic algae were collected (Ode et al., 2015). This information is provided for each sample in  $\text{cm}^2$ .

#### 4.1.4.2 Biovolume Calculations: SBA Quantitative Sample – Microalgal Fraction

**Step 1:** Calculate the biovolume of each microalgal NCE using the measured dimensions and formulae for geometric shapes closest to the cell's shape, proposed by Hillebrand et al. (1999) and specified for the SBA genera recorded by SWAMP (see Appendix E). For each



microalgal taxon, sum the biovolumes of all NCE recorded in the known number of analyzed optical transects.

This is a total biovolume of i-species ( $V_a$ ) in the analyzed optical transects in  $\mu\text{m}^3$ .

**Step 2:** This value ( $V_a$ ) needs to be corrected for the sample dilution, caused by addition of 5 mL of glutaraldehyde to the composite sample. The correction factor ( $V_{cr}$ ) is calculated as follows:

$$V_{cr} = (V_t - V_m) (V_t - V_m - 5)^{-1}$$

where:

$V_{cr}$  = a correction factor for sample dilution with fixative (assuming 5 mL of fixative was added to the sample)

$V_t$  = total initial sample volume in the sample vial (generally ~50 mL)

$V_m$  = volume of macroalgal fraction in the sample (will be 0 if no macroalgae detected)

Then to correct biovolume of i-species ( $V_a$ ) for fixative dilution use the following formula:

$$V_a' = V_a V_{cr} (\mu\text{m}^3)$$

**Step 3:** Use the following formula to calculate the biovolume of each microalgal species ( $\mu\text{m}^3$ ) per 1  $\text{cm}^2$  stream bottom area sampled. The result unit is  $\mu\text{m}^3 \text{cm}^{-2}$ .

$$V_i = V_a' V_s V_c^{-1} A^{-1} (\mu\text{m}^3 \text{cm}^{-2})$$

where:

$V_i$  = biovolume of i-species ( $\mu\text{m}^3$ ) per 1  $\text{cm}^2$  stream bottom area sampled

$V_a'$  = biovolume of i-species ( $\mu\text{m}^3$ ) per sample counted (known number of optical transects in which the enumeration has been done) corrected for the dilution with fixative ( $V_{cr}$ )

$V_s$  = composite sample volume (mL). It is the volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume typically does not exceed 400-500 mL (Ode et al., 2015). This information is provided for each sample in mL.

$V_c$  = volume of sample counted (mL) [this is the number of transects counted multiplied by the sample volume per transect and by dilution factor (see Note 5 below)];

$A$  = stream bottom area of substratum sampled. It is the total area of substratum surface from which benthic algae were collected (Ode et al., 2015). This information is provided for each sample in  $\text{cm}^2$ .

**Note 5:** The sample volume contained in one horizontal transect is determined as follows: a transect is a rectangular area of the slide in which the width is equal to the field of view and the length is equal to the length of the cover slip. With our microscope condition, at a 40x objective, the 0.55 mm width of the transect results in a cover slip (22 x 30 mm) consisting of 40 optical horizontal transects. For microscopes where the 40x field of view differs from 0.55 mm, calculate the transect width required.

Sample volume held by one horizontal optical transect is calculated as follows: on the counting slide, 0.05 mL of subsample is placed. This subsample has been concentrated 5 times the original sample, thus 0.25 mL from the original sample is analyzed. Therefore the original sample volume held by one horizontal optical transect is 0.00625 mL ( $=0.25 \text{ mL}/40$  horizontal transects).

When additional dilutions or concentrations are applied to the initial microalgal subsample of 1 mL (see Section 4.1.3 Step 2), the sample volume per transect must be corrected by multiplying with the dilution factor (DF). Most often, the subsample of 1 mL is counted without dilutions/concentrations (DF 1), but sometimes is concentrated to 0.5 mL (DF 2), or diluted to 2 mL (DF 1/2), to 3 mL (DF 1/3), to 4 mL (DF 1/4), to 5 mL (DF 1/5). Examples of microalgal biovolume calculation with our microscope are shown in Appendix F.

## 4.2 Identification and Enumeration Analysis of Diatoms

Identification of diatoms requires an understanding of how the cells are put together (each cell frustule being comprised of two valves and one to several girdle bands) and what frustule components are being identified and enumerated. The different views one may have of a frustule and/or its components is also important since attempts to key out specimens will require one to know the view in which one is seeing an individual cell. There are many fine structural elements of diatom cell walls and knowledge of this terminology is

imperative for the identification of species. Guides to this information can be found in the published literature, and commonly used books and floras include Patrick and Reimer (1966), Krammer and Lange-Bertalot (1986-1991), Round et al. (1990), and Kociolek et al. (2015 a, b) (see Section 1.3 and Appendix I). The fine structure of diatoms is the basis for the taxonomy of the group, and modern approaches to taxonomy are relying more and more on these fine structures to make effective distinctions that are not only reflected at species level, but also at the genus level.

**Step 1:** Position the slide on the microscope stage with its label to the right. Scan slide at medium magnification (200x or 400x) to confirm that diatoms are evenly distributed on the cover slip and to assess the taxonomic composition of the sample to be analyzed.

**Step 2:** Establish a horizontal transect for counting by positioning the 100x objective a short distance from the edge of slide, where valves are no longer optically distorted. A transect is a rectangular area of the slide in which the width is equal to the field of view and the length is equal to the length of the cover slip.

**Step 3:** Identify and enumerate all complete and partial valves visible in the first field of view. A partial valve is defined as having more than 50% of the valve including the central area. The valve (both complete and partial) must extend at least halfway into the transect, and must include the center of the valve in the transect. Once the diatoms in the first field of view have been enumerated move on to the next field of view in the direction of the horizontal transect. If a second transect needs to be counted, move to the first field of view of the second transect.

**Step 4:** Record the first and last field of view for each counted transect and the upper right corner of the cover slip by taking the coordinates from the microscope stage. Enter the coordinates in the *ID datasheet for Diatom Sample* (Appendix C2).

**Step 5:** Record each diatom taxon identified and the corresponding number of valves enumerated in the *ID Datasheet for Diatom Sample-Heading: Diatom taxon ID; Number of valves* (Appendix C2).

**Step 6:** Identify and enumerate 600 diatom valves across a known length of horizontal optical transects. Avoid counting valves in any disrupted areas of the mount, particularly

edges that have optical aberrations. When the diatom enumeration is completed, record the last field of view counted by taking the coordinates from the microscope stage. The last field of view counted can be located at any transect point. If the sample is very sparse, continue counting for 4 hours or until 300 valves are enumerated (whichever comes first), excluding time spent learning new species.

Record the number of transects traversed and the coordinates of the last field of view counted in the *ID Datasheet for Diatom Sample-Heading: Number of transects counted: xx* (Appendix C2).

**Step 7:** Take sufficient photomicrographs of all newly recorded species to support harmonization of results. Take photomicrographs of previously reported species to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

#### 4.3 Sample Labeling and Archiving

All SBA and diatom samples and slides must be retained as voucher specimens until harmonization and reporting of data is complete. All samples will be archived and stored as reference collections. Archives of samples and slides should be retained by the laboratory for two years. The following types of samples will be archived:

##### **SBA**

- Vials with qualitative SBA sample fixed with 2% glutaraldehyde
- 15 mL or 50 mL graduated centrifuge tube with minimally disturbed SBA macroalgal fraction fixed with 2% glutaraldehyde
- 15 mL graduated centrifuge tube with remaining SBA microalgal fraction fixed with 2% glutaraldehyde
- 50 mL graduated centrifuge tube with remaining content of original SBA sample fixed with 2% glutaraldehyde
- Sealed semi-permanent slides with analyzed quantitative SBA microalgal fraction

##### **Diatoms**

- Vials with unused cleaned diatom sample fixed with ethanol to 50% final concentration

- 50 mL graduated centrifuge tube with remaining content of original diatom sample fixed with 1% formalin
- Permanent diatom slides

All sample IDs in the archive follow the original SWAMP sample IDs, sampling date (MM/DD/YYYY), type of sample (see below). The fixed SBA samples should be kept dark and cool. Archived slides and samples must be retained by the laboratory until harmonization and reporting of data is completed.

#### **4.3.1 Archiving of SBA – Qualitative Samples**

##### **Materials needed:**

- Plastic scintillation vials (20-40 mL)
- 2% glutaraldehyde
- Parafilm

**Step 1:** Select a representative subsample that contains all identified macroalgal taxa and the algal material from the slides. Place the material in plastic scintillation vial, fix it with 2% glutaraldehyde final concentration and parafilm the cap.

**Step 2:** Labeled the vial by SWAMP sample ID, sampling date (MM/DD/YYYY), and note “qualitative sample” or “Q”.

#### **4.3.2 Archiving of SBA – Quantitative Samples**

##### **Materials needed:**

- Nail polish
- Slide box
- Parafilm

##### ***Macroalgal fraction***

**Step 1:** Place the entire content of the macroalgal fraction which has been analyzed back in the 15 mL or 50 mL tube, including the macroalgae investigated on microscope slide under compound microscope. This should be done very careful preserving the entirety of the sample.

**Step 2:** Refix the sample with glutaraldehyde to 2% final concentration and parafilm the

cap. Label the tube by SWAMP sample ID, collection date (MM/DD/YYYY), and note “macroalgae”.

### ***Microalgal fraction***

**Step 1:** When finished with taxonomic work and enumeration, seal the cover slip with nail polish, label the microscopic slide by SWAMP sample ID, collection date (MM/DD/YYYY), and note “microalgae”, and keep it in a slide box.

**Step 2:** Refix the subsample with 2% glutaraldehyde final concentration, parafilm the cap, and keep it separately from original sample for reference purposes. Label it by SWAMP sample ID, collection date (MM/DD/YYYY), and note “microalgae”.

### **4.3.3 Archiving of Diatoms**

#### **Materials needed:**

- Plastic scintillation vials (20 mL)
- Slide box
- 70% Ethanol
- Parafilm

**Step 1:** Label each slide by SWAMP sample ID, collection date (MM/DD/YYYY), and note “diatoms”, and keep it in a slide box.

**Step 2:** The remaining cleaned diatom material is kept in a vial with small amount of water and fixed with ethanol to 50% final concentration. Label each vial by SWAMP sample ID, collection date (MM/DD/YYYY), and note “diatoms”. This material can be used for additional light microscope or scanning electron microscope observations.

## **Section 5: Quality Assurance and Quality Control**

This SOP has been created to provide a method for generating SWAMP algal taxonomy data. In addition, this document represents the initial attempt at establishing quality assurance (QA) and quality control (QC) activities for algal taxonomic analysis.

### **Section 5.1 Laboratory Quality Control**

The procedures covering preparation and taxonomic analysis of diatoms are adapted from established methods. The QC procedures historically employed for diatoms subject a percentage of the permanent slides from a sample batch to identification and enumeration by a second taxonomist. QC assessment is achieved by calculation of the Percent Taxonomic Agreement for each pair of results, which is validated against an associated Measurement Quality Objective. These protocols are not included in current SOP. However, QC procedures that include validation by a secondary taxonomist, both internally and externally, are being discussed for inclusion in future versions. The primary QC focus is currently on taxonomic harmonization of the dataset. Harmonization of data has been identified as a valuable tool for improving data quality and consistency.

### **Section 5.2 Laboratory Quality Assurance**

The procedures outlined below work to strengthen the QA activities related to algal taxonomic analysis. The experience and knowledge of the taxonomists and laboratory technicians generating data using this SOP have an enormous impact on the results. Although appropriate QC measures are not yet developed, QA procedures designed to support generation of algal taxonomy data of known and documented quality are included. These include:

- Requiring laboratory documentation of taxonomist qualifications and training of new staff.
- Establishing sample handling requirements to ensure information vital to performing data assessment is properly and consistently documented and included in the meta-data.
- Requiring collection of photomicrographs, with guidelines for determining the appropriate amount of documentation needed.
- Requiring external taxonomic harmonization of the results generated using these procedures.

### **Section 5.3 Sample Handling Requirements**

#### **Section 5.3.1 Sample Handling Requirements – Point of Receipt**

Proper execution of identification and enumeration procedures requires algae samples in good condition. Poor sample handling can lead to degradation of algae samples and

interfere with taxonomic analysis and enumeration. To ensure sample condition is consistently maintained and documented, samples must meet the sample handling requirements in Table 1. The required corrective actions are listed in Table 2.

Sample	Container	Preservation	Required Hold Time
<b>SBA Qualitative Sample</b>	100 mL Whirl-Pak® bag	Sample stored cold (4°C) from time of collection to laboratory receipt. Sample <b>must not be frozen</b> at any point.	14 days at 4°C
<b>SBA Quantitative Sample</b>	50 mL centrifuge tube	Sample stored cold (4°C) from time of collection to preservation.	Samples must be preserved with 2% glutaraldehyde within 4-days of collection.
<b>Diatom Sample</b>	50 mL centrifuge tube	Preserve with 2% formalin as soon as possible.	N/A

**Table 1. Required Sample Conditions for Laboratory Receipt.**

Incident	Corrective Action
SBA Qualitative Sample received more than 14-days after collection, or has been frozen prior to receipt.	1) Taxonomist must inspect sample to evaluate and document condition on COC upon receipt. 2) Results must be flagged to indicate the missed hold time.
SBA Quantitative Sample preserved more than 4-days after collection.	1) Taxonomist must inspect sample to evaluate and document condition on COC upon receipt. 2) Results must be flagged to indicate the missed hold time.
SBA Quantitative Sample preserved, but with total volume less than 50 mL.	1) Taxonomist must inspect sample to evaluate and document condition on COC upon receipt. 2) Results must be flagged to indicate the reduced total sample volume.
Diatom Quantitative sample not preserved following collection.	1) Taxonomist must inspect sample to evaluate and document condition on COC upon receipt. 2) Results must be flagged to indicate the missed hold time.

**Table 2. Required Corrective Actions for Laboratory Receipt.**



### Section 5.3.2 Sample Handling Requirements – Archiving

This procedure establishes requirements for archiving the SBA and diatom samples. All samples must be archived by the laboratory until results have been harmonized and reported. Beyond this, the following guidelines are recommended:

- The SBA qualitative sample can be archived for a maximum of 28-days after the collection if kept cold (4°C).
- All other samples are preserved prior to archiving, and can be held for at least 2-years.

### Section 5.4 Photomicrographic Documentation Requirements

Requirements related to photomicrographic documentation are specified throughout the procedure as follows:

Take sufficient photomicrographs of all newly reported species to support harmonization of results. Take photomicrographs of previously identified taxa to demonstrate the key aspects of vegetative morphology and reproduction used in identification (see Section 1.5 and Appendix H).

At this point, SWAMP has not included a specific number of photomicrographs in this requirement. The number of images needed to support identification and harmonization may vary greatly; establishment of a minimum number would result in collection of too many photos in some instances and too few in others. It is therefore left to the judgment of the experienced taxonomist to determine how many photomicrographs need to be collected. The standard used to evaluate the photomicrographic documentation is whether or not it supports the stated goals:

- Photomicrographic documentation of newly reported species is sufficient to support harmonization of results.
- Photomicrographic documentation of previously reported species is sufficient to demonstrate the key aspects of vegetative morphology and reproduction used in identification.

Recommendations for producing high-quality photomicrographs are included in Appendix H. Photomicrographs displayed on online resources, [Soft-Bodied Stream Algae of California](#) and [Diatoms of the Southern California Bight](#), set the quality standard for

photomicrographs. Questions regarding photomicrographic documentation should be discussed with the algal taxonomist from CalPAL conducting harmonization of the results.

### **Section 5.5 Requirements for External Harmonization of Taxonomic Results**

A fully detailed procedure covering harmonization of taxonomic results is currently being developed (see Section 1.7). With respect to these procedures, laboratories should be aware that participation in external harmonization of results is required for SWAMP projects.

Harmonization requires communication between laboratory and CalPal taxonomists. This is achieved in part by laboratory submission of the photographic documentation and text descriptions of SBA and diatoms. Sometimes exchange of samples between the taxonomists is needed. While this process is time consuming for both taxonomists, it is critical to assuring the quality and comparability of the SWAMP algal taxonomy data and participation in this process is therefore required by SWAMP.

Taxonomic harmonization currently is focused on newly recorded algal names which have to be approved by an external taxonomist before the data is reported to the SWAMP database. Thus, harmonization should take place with enough time before the deadline for data reporting. It is recommended for new taxa recorded from the fresh SBA qualitative samples to be harmonized during the identification process, because unpreserved samples allow the best observation of taxonomic features and can be used for molecular analysis.

## **Section 6: Reporting of Algal Taxonomy Results**

### **6.1 Reporting of SBA Results**

All results collected for SBA sample (qualitative sample, quantitative sample - macroalgal fraction, quantitative sample - microalgal fraction) are entered into the *ID Datasheet for SBA Sample* (Appendix C1). Each taxonomic entry must have a corresponding numerical entry (e.g., counts and cell size measurements) with the exception of the qualitative sample which is not enumerated. After all data have been entered for a given sample, the numeric entries for all NCEs should be summed to ensure the total target count has been reached. SBA biovolumes should be calculated and entered in *Biovolume Calculation Datasheet*

(Appendix F). Taxon names entered onto the SWAMP Taxonomy Results template should match the SWAMP Algae Master Taxa list. Any new FinalID name generated from an analysis and confirmed during the taxonomy harmonization process should be added to the Organism\_DetailLookUp sheet in the SWAMP Taxonomy Results template and submitted to CalPAL for approval (see Sections 1.4 and 6.3, and Appendix G).

## 6.2 Reporting of Diatom Results

All data from the diatom identification and enumeration are entered into the *ID Datasheet for Diatoms* (Appendix C2). Each taxonomic entry must have a corresponding numerical entry (i.e., counts). After all data has been entered for a given sample, the numeric entries for counts should be summed to ensure the total target count has been reached.

Taxon names entered onto SWAMP Taxonomy Results template must match the SWAMP Algae Master Taxa list. Any new FinalID name generated from an analysis and confirmed during the taxonomy harmonization process should be added to the Organism\_DetailLookUp sheet in the SWAMP Taxonomy Results template and submitted to CalPAL for approval (see Section 1.4 and 6.3., and Appendix G).

## 6.3 Data Management and Reporting

The primary way for submitting algae taxonomy data is through the Microsoft Excel SWAMP Taxonomy Results template (Taxa Analysis Authorization or Taxa AA form) found on the SWAMP website under the Database Management Resources Templates page [http://www.waterboards.ca.gov/water\\_issues/programs/swamp/data\\_management\\_resources/templates\\_docs.shtml#taxonomy](http://www.waterboards.ca.gov/water_issues/programs/swamp/data_management_resources/templates_docs.shtml#taxonomy). Template documentation explaining specific data fields, business rules, and how to complete this file can be found at this link as well.

The procedure for using the template to report results is included in Appendix G. The procedure focuses on three tabs: AlgaeInfo, BenthicResults, and Organism\_DetailLookUp. It is important to understand which party (project management, field crew, or laboratory) is responsible for populating specific data within each tab. View the SWAMP and CEDEN online LookUp lists for the most current valid values for given fields.

The AlgaeInfo tab contains composite sample volume information for each sample

necessary for SBA biovolume calculations. The project manager (PM) and/or field crew is responsible for populating and providing this information to the taxonomy lab prior to completion of the data file.

The BenthicResults tab is used by the laboratory to report algae taxonomy results. The tab has three sections used for aligning and reporting data.

The first section (columns A through Y) details Sample through Collection information for each sample. This information is provided by the PM and/or field crew. Column T contains the data for stream bottom area of substratum sampled for each sample necessary for SBA biovolume calculations. The Chain of Custody (COC) contains some of the information within this section but it usually does not provide all of the necessary information.

The second section (columns Z through AL) contains the lab effort/sorting information for each sample. This information is populated by the taxonomy lab.

The third section (columns AM through BB) includes the taxonomy and numerical results for each FinalID name and LifeStageCode combination for each sample. This information is populated by the taxonomy lab. LifeStageCode can be found in separate tab: LifeStageLookUp.

It is important to be able to link the second and third sections with the first section to report the data to SWAMP or CEDEN. It is preferable for the taxonomy lab to receive the first section before data is reported so data can be aligned. If this does not occur, the LabSampleID can be used to link the taxonomy data with the sample information from the COC.

The Organism\_DetailLookUp tab is used for reporting FinalID names not currently listed on the Algae Master Taxa list. Each new FinalID name, including attribute information, should be submitted to CalPAL for review and approval before the Taxonomy Results template can be finalized (see Section 1.7).

After taxonomy harmonization is completed, results that need to be revised to reflect the outcome of harmonization are updated. The final data is added to the Taxonomy Result template BenthicResults tab in columns Z through BB aligning the data with the

corresponding Sample Collection information in columns A through Y. The data file is submitted by the taxonomy lab through an online checker to check for errors associated with formatting and business rules. SWAMP and CEDEN maintain their own checkers, thus, be sure to visit the appropriate website depending on where your data should be submitted as directed by the PM. The taxonomy lab should maintain an archive of all submitted data files.

## TERMS AND DEFINITIONS

**Algae.** Photosynthetic aquatic organisms, including soft-bodied algae and diatoms with uncovered reproductive structures;

**Akinete.** Thick-walled resting spore formed by transformation of a vegetative cell (John et al., 2011);

**Aplanospore.** Non-motile spore and not morphologically identical to mother cell (John et al., 2011);

**Benthic algae.** Bottom-living algae attached to or resting on the stream bottom (John et al., 2011), in contrast to planktonic algae which are free-floating in the water column;

**Biovolume.** Volume of biological material being measured;

**Chloroplast.** Double-membrane bound organelle in eukaryotic algae containing chlorophyll and other pigments (John et al., 2011);

**Cocoid.** Single-celled and non-flagellated algae;

**Coenocyte.** Thallus containing many nuclei, with few or no cell walls (John et al., 2011);

**Colony.** Group of individual cells enclosed into a common sheath/envelope or joined together and having characteristic form and structure (John et al., 2011);

**Conjugation.** In zygnematalean algae a process of fertilization of two gametes either within a tube which develops between two filaments in which the gametes were formed, or in the cells of the filament;

**Composite sample volume.** Volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume should not exceed 400-500 mL (Ode et al., 2015, Glossary, and p. 27). This information is provided for each sample;

**Cyanobacteria (cyanoprokaryotes, blue-green algae)** are photosynthetic prokaryotes,

that is, cells that have no membrane-bound organelles, including chloroplasts. For the purposes of this SOP, cyanobacteria are subsumed under SBA.

**Diatom.** A unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a “pill box” (Ode et al., 2015);

**Epiphytic algae.** Growing on another plant, including another alga (John et al., 2011);

**Filament.** Cells united or arranged in one or more rows to form a chain or thread (John et al., 2011);

**Frustule.** The complete silicified cell-wall of diatoms, consisting of the epi- and hypotheca (Krammer and Lange-Bertalot, 2000);

**Girdle.** Collective term for all structural elements between two diatom valves (Krammer and Lange-Bertalot, 2000);

**Heterocyst (or heterocyte).** A thick-walled, multilayered, and weakly pigmented cell in certain cyanobacteria, contains the nitrogenase enzyme, which enables fixation of gaseous nitrogen to ammonium (John et al., 2011);

**ID datasheet.** Excel spreadsheet file filled out on the computer that contains all algal taxa with corresponding counts and size measurements;

**Macroalgae.** Large macroscopic filamentous, colonial, tuft-forming, crustose, tissue-like or coenocytic eukaryotic algae and cyanobacteria that have forms recognizable with the naked eye (e.g., *Nostoc*, *Rivularia*, *Batrachospermum*, *Lemanea*, *Cladophora*, *Draparnaldia*, *Oedogonium*, *Rhizoclonium*, *Spirogyra*, *Zygnema*, *Mougeotia*, *Vaucheria*) [see Sheath and Cole (1992) for definitions of forms];

**Microalgae.** Small microscopic form not recognizable with the naked eyes but consisting of unicellular, colonial or filamentous non-diatom algae;

**Natural counting entity (NCE).** Each natural occurring form of algae (i.e., each unicell, colony, filament, tissue-like form, coenocyte, tuft, or crust) is defined as a natural counting entity regardless of the number of cells in the thallus or colony;

**Pyrenoid.** Organelle associated with the chloroplast of many algae, which contains a high content of enzyme responsible for fixing carbon dioxide in photosynthesis and around which starch grains may accumulate (John et al., 2011);

**Relative abundance of diatoms.** The number of valves of particular diatom taxon as a percentage of the total number of diatom valves counted per sample (which is at least 600 diatom valves in this SOP);

**Soft-bodied algae (SBA).** Non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are subsumed under this assemblage (Ode et al., 2015);

**Stream bottom area of substratum sampled.** Total area of substratum surface from which benthic algae were collected (Ode et al., 2015, pp. 10-18). This information is provided for each sample in cm<sup>2</sup>;

**Thallus (Thalli).** Body of simple plants not differentiated into a true root, stem, leaf or leaves (John et al., 2011);

**Tuft.** Group of filaments which are close together but without a common sheath, usually aggregations at right angles to a surface (John et al., 2011);

**Zygospore.** Spore formed sexually following gamete fusion, often thick-walled, characteristically colored and sometimes ornamented (John et al., 2011);

**Valve.** Lid-top of the epi-and hypotheca in diatom. It consist of the valve face and that part of the valve, that connects with the girdle (Krammer and Lange-Bertalot, 2000).



# LIST OF APPENDICES

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**Appendix E: List of Geometric Shapes Applied in Biovolume Calculation for SBA**

**Appendix F: Biovolume Calculation Datasheet for SBA Microalgal Fraction with  
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**Appendix G: Protocol for the SWAMP Database Reporting**

**Appendix H: Recommendations for producing high-quality algal photomicrographs**

**Appendix I: References**

## Appendix A: Laboratory Equipment List

Items	Supplier and Catalog #	SBA	Diatoms
<b>Sample Processing</b>			
Beakers, 50 mL	Fisher Scientific, 07-250-053	x	
Beakers, 250 mL	Fisher Scientific, 07-250-056	x	x
1.5 mL eppendorf tube	Fisher Scientific, 05-408-129	x	
10 mL pipette		x	x
146 mm borosilicate pipette	Fisher Scientific, 13-678-20A	x	x
15 mL and 50 mL graduated centrifuge tube	Fisher Scientific, 05-538-59A & 07-203-510	x	x
Bemis Parafilm "M"	Fisher Scientific, 13-374-12	x	
Dissecting needles	Fisher Scientific, S97397	x	x
Forceps, 30 cm long	Fisher Scientific, 10316C	x	x
Frosted glass microscope slides	Fisher Scientific, 12-550-343		x
Glass specimen dish, 120 mm x 54 mm	Forensics Lab supply, 5541170	x	
Glass cover slips - 18 x 18 mm or 22 x 22 mm , No. 1 thickness, stored in covered glass jar filled with 70% ethanol	Fisher Scientific, 12548A or 12-548-B		x
Graduated cylinders		x	
Grid bottom culture dishes, 70 and 100 mm diameter	Chemglass Life Sciences, CLS-1806-01 & CLS-1806-02	x	
Hot plate with temperature control			x
Jewelers micro forceps #4	Ted Pella Inc, 524	x	
Labels		x	x
Laboratory coat		x	x
Micro-centrifuge		x	
Microspatula			x
Nail polish	CVS Pharmacy	x	
Nitrile gloves	Genesee Scientific, 44-100M	x	x
pH paper			x
Plastic scintillation vials (20-40 mL) for storing samples (see recommendations in Appendix B1)	Research Products International Corp, 121043	x	x
Protective eyewear		x	x
Scissors			x
Slide box	Fisher Scientific, 03-446	x	x
Squirt bottle of DI water		x	x
Table-top centrifuge		x	x
Vortex		x	

Identification and Enumeration			
Research quality trinocular dissecting microscope		x	
Research quality light trinocular compound microscope (with 4x, 10x, 20x, 40x, 100x objectives), ocular micrometer calibrated for each objective. DIC or phase contrast optics are helpful, but optional		x	
Research quality light trinocular compound microscope (with 10x, 40x, 63x oil and 100x oil immersion objectives, an immersed condenser lens), ocular micrometer calibrated for each objective, and DIC optics			x
Digital camera connected to trinocular head with computerized software for measuring		x	x
Digital imaging software and computer		x	x
2.5x and 5x photoeyepiece (necessary if camera tube is required for digital camera of choice)		x	x
Frosted glass microscope slides	Fisher Scientific, 12-550-343	x	
Glass cover slips - 22 x 30 mm	Fisher Scientific, 12-548-5A	x	
Immersion oil	Fisher Scientific, NC0379246		x
Lens paper		x	x
Chemicals			
Concentrated hydrogen peroxide	Fisher Scientific, S25360		x
Concentrated nitric acid	Fisher Scientific, S25449A		x
Ethanol 70%	Fisher Scientific, A407P-4		x
Formalin 10%	Fisher Scientific, SF98-4		x
Glutaraldehyde 50% histological grade stock solution	Fisher Scientific, BP2547-1	x	
Lugol's Iodine Solution	Carolina Biological Supplies, 87-2795	x	
Naphrax	PhycoTech Inc, P-Naphrax15		x
Potassium dichromate	Electron Microscopy Sciences, 20100		x
Toluene	Fisher Scientific, T324-500		x

## **Appendix B: Health and Safety Issues**

### **Appendix B1: Health and Safety Issues for SBA Processing**

As with all laboratory procedures, care should be given when working with glass and any chemicals. Specific safety issues in the laboratory related to SBA processing is to work safe with samples which are fixed with 2% glutaraldehyde. These samples include all quantitative algal samples collected and fixed in the field, as well as the vials with preserved and stored qualitative algal samples as reference collections. All procedures with samples fixed with 2% glutaraldehyde are to be performed in a positive-draw fume hood. Staff members should wear laboratory coats, nitrile gloves and protective eyewear. When fixed qualitative samples are reinvestigated, the algal material should be washed with DI water prior to microscopic examination by soaking in beakers with DI water for 2 hours. Processing of quantitative algal samples includes a dilution of 2% glutaraldehyde in the sample by at least 20x (see Section 3.1.2 and 3.1.3).

### **SOP for using glutaraldehyde for the preservation of SBA**

#### **1. Scope and Application**

Glutaraldehyde is a colorless liquid with a pungent odor used as a fixative. This SOP covers the use of glutaraldehyde by SWAMP laboratories as a fixative for SBA.

#### **2. Health Hazards**

The health hazards associated with the use of glutaraldehyde include:

##### **Inhalation**

- Regulatory limit of 0.05 ppm as a ceiling level
- Chemical burns to the respiratory tract
- Asthma and shortness of breath
- Headache, dizziness, and nausea

##### **Skin**

- Sensitization or allergic reactions, hives
- Irritations and burns
- Staining of the hands (brownish or tan)

**Eyes**

- Irritation and burns. Eye contact causes moderate to severe irritation, experienced as discomfort or pain, excessive blinking and tear production
- May cause permanent visual impairment
- Conjunctivitis and corneal damage

**Ingestion**

- Gastrointestinal tract burns
- Central nervous system depression, excitement
- Nausea, vomiting
- Unconsciousness, coma, respiratory failure, death

**3. Safety Shower and Eyewash**

All employees using glutaraldehyde must be aware of the location and use of the laboratory safety shower and eyewash, and must be able to reach it within 10 seconds from the time of contamination. At no time will processes using glutaraldehyde be allowed that do not provide access to a safety shower and eyewash.

Employees who have skin or eye contact with glutaraldehyde will immediately stop all processes and proceed to the safety shower and eyewash station. The employee will rinse the affected area for a minimum of 15 minutes. If eye contact has occurred, the upper and lower eyelids must be lifted to allow adequate flushing of the eyes.

**4. Special Handling Procedures and Storage Requirements**

Procedures will be followed that reduce exposure to glutaraldehyde vapor to the lowest reasonable level.

This includes:

- Ensure glutaraldehyde is only used under a positive-draw fume hood.
- Use only enough glutaraldehyde to perform the required procedure.
- Every effort must be made to minimize splashing, spilling, and personnel exposure
- Once specimens are preserved, they will be capped or secured in a way that does not allow glutaraldehyde to evaporate into the lab.
- At no time will open containers be removed from the positive-draw fume hood.

- All containers of glutaraldehyde or solutions containing glutaraldehyde will be appropriately marked with the chemical name, and hazard warning label.
- Glutaraldehyde will be stored in tightly closed containers in a cool, secure, and properly marked location.
- Glutaraldehyde storage vials should meet the following safety requirements: If they are plastic - high-density Polyethylene (HDPE) will be one of the best plastic not corroded by the glutaraldehyde. Low-density Polyethylene (LDPE) loses some of its capabilities at temperatures higher than 50°C. Polypropylene (PP) works excellently just as well. For any scintillation vial, no metal/foil liners should be inside the cap. Borosilicate glass vials or any polypropylene copolymers will work well also. No flexible PVP (Polyvinylpyrrolidone) or PMP (polymethylpentene) should be used.

## 5. Waste Disposal

Excess glutaraldehyde and all waste material containing glutaraldehyde must be placed in an unbreakable secondary container labeled with the following “HAZARDOUS WASTE GLUTARALDEHYDE.” Wastes will be disposed of through the laboratory hazardous waste contract.

## Appendix B2: Health and Safety Issues for Diatom Processing

As with all laboratory procedures, care should be given when working with glass and any chemicals. For all chemicals, the laboratory should maintain current files /documentation in the form of Material Safety Data Sheets (MSDS) and those files should be available to all laboratory staff. Should there be any concern, laboratory staff should take appropriate action (relative to their setting). Specific safety issues in the laboratory related to diatom processing include:

**Glassware.** There are many places in these procedures where glassware is used, including use of beakers for sample process, pipettes, microscope slides, and especially the thin cover glasses used. Breakage of glassware should be cleaned up immediately, with broken glass being disposed of in specific, marked containers.

**Oxidizers.** This includes nitric acid and 30% hydrogen peroxide. Laboratory personnel should have eye protection and wear gloves when using these materials. Should there be a

spill, spill kits should be used to contain and clean up the spill.

**Fixatives.** Formalin, the primary fixative used in the preservation of fresh diatom samples, requires care in handling and disposal. It should be handled only in a positive-draw fume hood, and disposal should be made in accordance with a company's hazardous waste disposals procedures and protocols.

**Naphrax/Toluene.** The solvent toluene is used to keep the mounting medium Naphrax as a liquid. It is evaporated when heated. In both its liquid and vapor forms, toluene can be hazardous to health, so it should be used exclusively in a positive-draw fume hood.

**Potassium dichromate.** This chemical is highly hazardous to health and environment. It is a strong oxidizer, corrosive, irritant, sensitizer, carcinogen, highly toxic acts upon eyes, skin, lungs, mucous membranes, reproductive toxin, fatal if inhaled. It should be used exclusively in a positive-draw fume hood and disposed of as hazardous material. Not recommended for use.

**Hot plates.** Just as their name indicates, use of hot plates means that care must be taken not to burn oneself.

## Appendix C. ID Datasheets

### Appendix C1: ID Datasheet for SBA Sample with Example Form

SWAMP sample ID, Sampling date						
Qualitative sample - List of taxa						
Macroalgal taxon ID						
Quantitative sample - Macroalgal fraction (sample volume: xx mL)						
Macroalgal taxon ID	Proportion of each taxon (%)					
Non-algal matter						
TOTAL						
Epiphyte taxon ID	# NCE					
TOTAL						
Quantitative sample - Microalgal fraction additional dilution/concentration to xx mL; dilution factor (DF) # horizontal transects counted: xx						
Microalgal taxon ID	# NCE	Cell width ( $\mu\text{m}$ )	Cell/Filament length ( $\mu\text{m}$ )	Cell depth ( $\mu\text{m}$ )	Total cell number	Total filament length ( $\mu\text{m}$ )
TOTAL						



## Example Form

907SDR035, 04/15/2015						
<b>Qualitative sample - List of taxa</b>						
<b>Macroalgal taxon ID</b>						
<i>Cladophora glomerata</i>						
<i>Oedogonium</i> sp 3						
<i>Rhizoclonium hieroglyphicum</i>						
Diatoms ( <i>Pleurosira</i> )						
<b>Quantitative sample - Macroalgal fraction (sample volume: 0.3 mL)</b>						
<b>Macroalgal taxon ID</b>	<b>Proportion of each taxon (%)</b>					
<i>Cladophora glomerata</i>	45					
<i>Oedogonium</i> sp 3	30					
<i>Rhizoclonium hieroglyphicum</i>	20					
Diatoms ( <i>Pleurosira</i> )	5					
Non-algal matter	0					
<b>TOTAL</b>	<b>100</b>					
<b>Epiphyte taxon ID</b>	<b>#NCE</b>					
<i>Chamaesiphon incrustans</i>	15					
<i>Chroococcopsis epiphytica</i>	9					
<i>Leptolyngbya foveolarum</i>	76					
<b>TOTAL</b>	<b>100</b>					
<b>Quantitative sample - Microalgal fraction</b>						
additional dilution to 2 mL; dilution factor (DF $\frac{1}{2}$ )						
# horizontal transects counted: 9						
<b>Microalgal taxon ID</b>	<b>#NCE</b>	<b>Cell width (<math>\mu\text{m}</math>)</b>	<b>Cell/Filament length (<math>\mu\text{m}</math>)</b>	<b>Cell depth (<math>\mu\text{m}</math>)</b>	<b>Total cell number</b>	<b>Total filament length (<math>\mu\text{m}</math>)</b>
<i>Chlorella vulgaris</i>	21	5.2*	6.8*		21	
<i>Chroococcus minimus</i>	29	2.1*	2.1*		125	
<i>Leptolyngbya foveolarum</i>	128	1.2*	60.7*			7769.6
<i>Oocystis pusilla</i>	7	4*	8.5*		26	
<i>Phormidium formosum</i>	72	5.1*	49.6*			3571.2
<i>Pteromonas</i> sp 1	1	15	21	9	1	
<i>Scenedesmus dimorphus</i>	33	3.9*	11.8*		124	
<i>Scenedesmus ellipticus</i>	24	4.1*	8.7*		96	
<b>TOTAL</b>	<b>315</b>					

**Note:** \* Indicates averaged dimensions based on measurements of 20 NCEs per species.

## Appendix C2: ID Datasheet for Diatom Sample

<b>SWAMP sample ID, Sampling date</b>	
<b>Diatom taxon ID</b>	<b>Number of valves</b>
<b>TOTAL</b>	
<b>Number of transects counted: (xx)</b>	
<b>Point of interest</b>	<b>Microscope stage coordinates</b>
Upper right corner of cover slip	
First field of view (first transect)	
Last field of view (first transect)	
First field of view (second transect)	
Last field of view (second transect)	
.....	
First field of view (last transect)	
Last field of view (last transect)	

## Appendix D. Taxonomic Harmonization Datasheet

The taxonomic harmonization datasheet is a modification of the Organism\_DetailLookUp tab in the SWAMP Taxonomy Results template (see Appendix G). The additional columns presented below are distributed in the Organism\_DetailLookUp tab after the Final ID column for each new SBA and diatom name.

Additional Columns	Explanation
SWAMP sample ID	Code designating where the taxon was collected
Sampling date	Date when the sample was collected
Sample type code	Macroalgae, Microalgae, or Epiphyte for SBA; Integrated for diatoms
Abundance	NCE for SBA, number of valves for diatoms
Photomicrograph filename*	Copied filename of the photomicrograph
Notes from primary taxonomist	Notes on algal morphology including size measurements
Comments from CalPAL taxonomist	Comments on the taxon, request for further action if needed
FinalID approved	Lowest taxonomic level FinalID approved

**Note:** \* Each photomicrograph should have a filename consisting of the following elements in the order indicated: SWAMP Sample ID, Sampling date (MM/DD/YYYY), Species ID, magnification of the objective (i.e., 40x) (see Section 1.5 and Appendix H).

## Appendix E: List of Geometric Shapes Applied in Biovolume Calculation for SBA

This list includes all geometric shapes (after Hillebrand et al., 1999) applied in biovolume calculation of stream SBA for the SWAMP program. Many stream SBA genera recorded in the SWAMP Master list and illustrated in the online identification tool Soft-Bodied Stream Algae of California (Stancheva et al., 2014) are missing in Hillebrand et al. (1999). They are listed below in the corresponding geometric shape category.

**Cylinder.** It is one of the most common shapes applied in the biovolume calculation of all filamentous algae and some coccoids with cylindrical cells. In the case of tapering filaments the diameter is averaged by measuring the cells in the widest, narrowest and middle part of the filament.

Cyanobacteria: *Anabaena*, *Capsosira*, *Chamaesiphon confervicola*, *C. incrustans*, *C. investiens*, *Dolichospermum*, *Trichormus*;

Chlorophyta: all green branched and unbranched filamentous genera, *Teilingia*;

Xanthophyta: *Xanthonema*

**Sphere.** Common shape applied in biovolume calculation of many flagellated and nonmotile unicells or colonial algae. The shape is applied to a single cell, and in the case of colony the biovolume of a single cell is multiplied by the total number of cells in the colony.

Chlorophyta: *Apiocystis*, *Asterococcus*, *Botryosphaerella*, *Harriotina*, *Radiococcus*, *Schizochlamys*, *Tetrasporidium*;

Chrysophyceae: *Chrysopyxis*;

Xanthophyta: *Mischococcus*

**Prolate spheroid.** Common shape applied in biovolume calculation of many flagellated and nonmotile unicells or colonial algae. The shape is applied to a single cell, and in the case of a colony, the biovolume of a single cell is multiplied by the total number of cells in the colony.

Cyanobacteria: *Coelomorion*, *Pleurocapsa*, *Chamaesiphon* species, excluding the species with cells approximated as cylinders

Chlorophyta: *Apatococcus*, *Chlamydomonadopsis*, *Comasiella*, *Nephrocytium*,

*Sphaerobotrys*, *Planotaenium*;

Chrysophyceae: *Stylococcus*;

Xanthophyta: *Chadefaudiothrix*, *Chlorosaccus*

Cryptophyta: *Chroomonas*

**Ellipsoid.** Chlorophyta: *Oocardium*

**Prism on elliptic base.** This shape refers to entire colony of *Pediastrum*, *Lacunastrum*, and *Stauridium* (Chlorophyta), where the transapical section height is approximated to 2  $\mu\text{m}$ .

**Two cones.** Chlorophyta: *Quadrigula*; Xanthophyta: *Chytridiochloris*

**Two truncated cones.** Chlorophyta: *Stauridium*

**Ellipsoid + cylinder.** Euglenophyta: *Monomorphina*, *Strombomonas*

More rarely applied shapes are cube, box, cylinder + two cones, cylinder+cone, ellipsoid+two cones+cylinder, half ellipsoid+cone on elliptic base.

## Appendix F: Biovolume Calculation Datasheet for SBA Microalgal Fraction with Example Form

Vc = Volume of sample counted (mL)			
	Sample volume per		
Transects counted (#)	transect (mL)	Dilution factor (DF)	Vc

Va' = Biovolume of i-species per sample counted ( $\mu\text{m}^3$ )						
Vt	Vm	Vt-Vm	Vt-Vm-5	Vcr	Va	Va'

Vi = Volume of i-species per unit bottom area ( $\mu\text{m}^3 \text{ cm}^{-2}$ )						
A	Vs	Va'	Vc	Va' Vs	Vc A	Vi

### Formulas (see Section 4.1.4.2)

- $Va' = Va \text{ Vcr} (\mu\text{m}^3)$ ,  $Vcr = (Vt - Vm) (Vt - Vm - 5)^{-1}$
- $Vi = Va' \text{ Vs } Vc^{-1} \text{ A}^{-1} (\mu\text{m}^3 \text{ cm}^{-2})$

### Sample and Microscope condition

- 1 mL microalgal fraction, 5 times concentrated original sample
- 0.05 mL subsample analyzed = 0.25 mL original sample
- Transect width with 40x objective = 0.55 mm
- 40 horizontal optical transects on the slide
- Sample volume per transect = 0.00625 (= 0.25/40) mL in 1 mL microalgal fraction
- Sample volume per transect should be corrected by multiplication with dilution factor (DF) when additionally concentrated to 0.5 mL (DF 2), or diluted to 2 mL (DF 1/2), to 3 mL (DF 1/3), to 4 mL (DF 1/4), or to 5 mL (DF 1/5)

### Example form for calculation of biovolume of i-species in two different sample conditions

**Sample 1:** Total biovolume of i-species in 3 horizontal optical transect analyzed is  $523 \mu\text{m}^3$  ( $V_a$ ); no additional dilutions/concentrations of microalgal fraction ( $DF = 1$ ); no macroalgae present ( $V_m = 0 \text{ mL}$ );  $V_t = 50 \text{ mL}$ ;  $A = 138.6 \text{ cm}^2$ ;  $V_s = 345 \text{ mL}$

**Sample 2:** Total biovolume of i-species in 3 horizontal optical transect analyzed is  $523 \mu\text{m}^3$  ( $V_a$ ); dilution of microalgal fraction to 2 mL ( $DF = 1/2$ ); 0.3 mL macroalgae present ( $V_m = 0.3 \text{ mL}$ );  $V_t = 50 \text{ mL}$ ;  $A = 138.6 \text{ cm}^2$ ;  $V_s = 465 \text{ mL}$

Vc = Volume of sample counted (mL)			
	Transects counted (#)	Sample volume per transect (mL)	Dilution factor (DF)
Sample 1	3	0.00625	1
Sample 2	3	0.00625	1/2

Va' = Biovolume of i-species per sample counted ( $\mu\text{m}^3$ )						
	Vt	Vm	Vt-Vm	Vt-Vm-5	Vcr	Va'
Sample 1	50	0	50	45	1.1111	581.1
Sample 2	50	0.3	49.7	44.7	1.1118	581.5

Vi = Volume of i-species per unit bottom area ( $\mu\text{m}^3 \text{ cm}^{-2}$ )						
	A	Vs	Va'	Vc	Va' Vs	Vi
Sample 1	138.6	345	581.1	0.01875	200479	77144.4
Sample 2	138.6	465	581.5	0.009375	270397	208097.7

## Appendix G: Protocol for the SWAMP Database Reporting

The following protocol for data entry and submittal to the SWAMP database is used by laboratories after SBA and diatom identification and enumeration data are entered in the ID datasheets (Appendix C), and SBA biovolumes are calculated (Appendix F). A similar process can be followed for submitting CEDEN data but the template may differ slightly.

The primary way for submitting algae taxonomy data is through the Microsoft Excel SWAMP Taxonomy Results template (Taxa Analysis Authorization or Taxa AA form) found on the SWAMP website under the Database Management Resources Templates page [[http://www.waterboards.ca.gov/water\\_issues/programs/swamp/data\\_management\\_resources/templates\\_docs.shtml#taxonomy](http://www.waterboards.ca.gov/water_issues/programs/swamp/data_management_resources/templates_docs.shtml#taxonomy)]. Template documentation explaining specific data fields, business rules, and how to complete this file can be found at this link as well. It is important to understand who is responsible for populating specific data within each template tab and when each task should occur.

Many tabs exist in the Taxonomy Results template, but only one (BenthicResults) is used to report algal taxonomy data. Most tabs are used for submitting new LookUp values for given data fields, but users should view the SWAMP and CEDEN online LookUp lists for the most current valid and comparable values. The Header row of each tab contains the corresponding field names for those data tables. Any field name in bold text indicates it is a required field and should be populated. If the value is unknown, default values may be used but it is preferable to use correct values. Three tabs will be discussed in greater detail in this document: AlgaeInfo, BenthicResults, and Organism\_DetailLookUp.

The AlgaeInfo tab contains sample volume information for each sample necessary for biovolume calculations. The Project Manager (PM) and/or field crew is responsible for populating and providing this information in the template file to the taxonomy lab prior to completion of the data file. If the physical habitat (PHAB) and sample collection data are entered into a SWAMP database (shell or replica), the SWAMP Bioassessment field forms can be used to run an existing query (Algae Sample Information) to export this data to the template file.



The BenthicResults tab is used by the lab to report SBA and diatom taxonomy results. The tab has three sections used for aligning and reporting data. The first section (columns A through Y) details Sample through Collection information for each sample and is to be provided by the PM and/or field crew. A COC contains some of the information within this section but it usually does not provide all of the necessary information. If the sample collection information exists in a SWAMP database (shell or replica), the PM or field crew can export this data using the SWAMP Bioassessment field forms by running the Taxa Template query. The second section (columns Z through AL) contains the lab effort/sorting information for each sample and is to be populated by the taxonomy lab. The third section (columns AM through BB) includes taxonomy results for each FinalID name and LifeStageCode combination for each sample, and it is to be populated by the taxonomy lab. It is important to be able to link the second and third sections with the first section to report data to SWAMP or CEDEN. It is preferable for the taxonomy lab to receive the first section before data is reported so data can be aligned. If this does not occur, a LabSampleID can be used to link the taxonomy data with the sample information from the COC.

The Organism\_DetailLookUp tab is used for reporting FinalID names not in the Algae Master Taxa list. Each new FinalID name, including attribute information such as taxonomic hierarchy and taxonomic authority, should be submitted to CalPAL for review and approval before the Taxonomy data template can be finalized. Additional supporting documentation such as photographs should also be submitted during the taxonomic harmonization process (see Sections 1.4 and 1.7).

After FinalID harmonization is completed, the final data is added to the Taxonomy Results template BenthicResults tab in columns Z through BB aligning the data with the corresponding Sample Collection information in columns A through Y. The data file is then submitted by the taxonomy lab through an online checker to check for errors associated with formatting and business rules. SWAMP and CEDEN maintain their own online checkers so please visit the appropriate web site depending on where your data should be submitted [SWAMP online checker: [http://swamp.waterboards.ca.gov/swamp\\_checker/](http://swamp.waterboards.ca.gov/swamp_checker/)]. The SWAMP online checker will ask for the Data Category (Taxonomy), your email address and agency, and for you to browse to your file for loading. After the file is checked, any associated errors will be shown on a Summary page where users can choose to hide errors

from showing if they want. Any critical errors must be fixed in the data file before it can be submitted to SWAMP. Any Regular or Warning errors should be reviewed and, if possible, fixed by either the taxonomy lab, PM, and/or field crew. When the green 'Submit Data to SWAMP' button is available, users click on the button to submit the file along with adding any comments and/or emailing other people with their submittal. Please note the entity responsible for submitting the final data set to SWAMP or CEDEN will depend on the project. That is, the PM may decide the taxonomy lab is to submit the data directly through the online checker, or the PM may want the taxonomy lab to submit the file to the PM first and the PM will be responsible for running the file through the online checker for submittal. In either case, the taxonomy lab should maintain an archive of all final data files whether it is submitted directly to the PM, SWAMP, or CEDEN.

The database protocol in this SOP is current as of the publication date but is subject to change as updates are made to SWAMP and CEDEN. Always confirm the current protocol with the SWAMP or CEDEN Data Management team and/or your PM.

## Appendix H: Recommendations for producing high-quality algal photomicrographs

### **Soft-Bodied Macroalgae**

1. Use a clean slide and cover slip to prepare water mount.
2. Select specimens, either fresh or preserved, which are representative and in good shape. If the specimen which has to be pictured is already mounted in an overcrowded slide, try to isolate it and move it to another slide. Overcrowded slides will create too many depths of view and overlap for clear 2D imaging.
3. Prepare a water mount with the algal specimen spread out well enough so that it lies flat on the slide. Avoid breaking or overlapping filaments or other structures. Avoid using too much or too little water to prepare the slide; too much will flood the slide and create depths of view or bubbles that can overlap the image, while too little will often cause plasmolysis of the sample.
4. Large mucilaginous cyanobacterial colonies should be gently flattened with the cover slip to make sure the cells are visible. Large red algae, such as *Batrachospermum*, should be gently flattened with cover slip to disclose the reproductive cells, and chopping with a razor blade may be necessary. Protect large spores of Zygnemataceae, *Oedogonium*, *Vaucheria* from breaking while mounting the material.
5. When the macroalga is mounted properly in a flat, single-cell layer, adjust the background light and white balance, and take a picture at lower magnifications (10x, 20x objective) to show the gross morphology of the filaments or colonies.
6. Take additional pictures at higher magnification (40x objective) focused on critical taxonomic features, such as akinetes and mucilage in cyanobacteria, chloroplast, type of branching, specialized cells, reproductive cells and spore cell walls in filamentous eukaryotic algae, etc.
7. Stain the cells with Lugol's solution if needed to distinguish the starch and take additional pictures.

### **Soft-Bodied Microalgae**

1. Use clean slide and cover slip to prepare water mount.
2. Place a drop of microalgal sample on the slide and avoid the formation of air bubbles while covering with the cover slip.
3. Make sure the sample is not too dense and the cells are not overlapping.

4. For photographing specimens, whether filaments, colonies or single cells, select those which lie flat on the slide. Press or tap the cover slip gently to adjust the position of the specimen if needed.
5. When the microalga is mounted properly in a flat, single-cell layer, adjust the background light and white balance, and take pictures at higher magnifications (40x objective) focusing on critical taxonomic features, such as chloroplasts, pyrenoids, cell walls, mucilage, etc.
6. Stain the cells with Lugol's solution if needed to distinguish the starch and take additional pictures.

### **Diatoms**

1. Make sure that the permanent slide and cover slip are clean before starting.
2. Select a valve which lies flat on the slide in valve view.
3. Avoid photographing valves which are tilted or covered by debris, in clumps or broken.
4. Avoid photographing valves located in disrupted areas of the mount, particularly edges that have optical aberrations.
5. When the diatom valve of interest is selected, adjust the background light and white balance, as well as the DIC with the 100x oil objective.
6. Focus on the surface of the valve so that the gross morphology, i.e. valve shape and structural elements are clearly visible.
7. Take additional pictures of the same valve focusing on critical taxonomic features, such as stigma in the central area in *Gomphonema*, pseudoceps and longitudinal lines in *Gomphoneis*, annulae at the poles in *Geissleria*, central nodule and fibulae in *Nitzschia*, central area and striae in *Navicula*, etc.

## Appendix I: References

### General References

- Acker, F., Russell, B., and E. Hagan. 2002. Diatom Cleaning by Nitric Acid Digestion with a Microwave Apparatus. Protocol P- 13-42. In: Charles, D.F., C. Knowles, and R.S. Davis, eds. 2002. 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, Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA. 124 pp. Also at <http://diatom.ansp.org/nawqa/pdfs/ProtocolPublication.pdf>
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### **Taxonomic Identification Literature and Online Resources**

**\* Indicates the references to start identification, including most recent taxonomic publications on the local algal flora, and then to expand the search to references for particular algal groups**

### **References for Identification of SBA**

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