### **Measurement Quality Objectives for Algae Taxonomy**



The following Measurement Quality Objectives (MQOs) establish guidelines for field sample collection and handling, and laboratory analyses of soft-bodied algae and diatoms. Guidelines contain both requirements and recommendations for SWAMP and participating SWAMP-comparable bioassessment projects. \*See <u>glossary table</u> (page 7) for definitions of acronyms and terms

#### Table 1. MQOs for Field Sample Collection, Handling and Processing of Algae

Analyte	Container	Preservation	Temperature & Holding Time
Chlorophyll a	Glass-fiber filter	No additives	Filter, wrap in foil, store on wet ice in the field, but freeze (pref80°C) within 4 h of collection; analyze within 28 d
Ash free dry mass (AFDM)	Glass-fiber filter (pre-combusted)	No additives	Filter, wrap in foil, store on wet ice in the field, but freeze (pref80°C) within 4 h of collection; analyze within 28 d
SBA quantitative sample	50 mL centrifuge tube	Add glutaraldehyde (to a 2% final concentration) under a fume hood, as soon as possible, but no later than 96 hours after sampling. Samples can arrive to the lab preserved, or unpreserved if received the day after sampling and lab is alerted in advance	Keep samples in dark on wet (not dry) ice. Keep at 0-4°C; do not freeze. After fixing, refrigerate (0-4°C) and keep in dark; fixed samples can be stored for at least 2 years
SBA qualitative sample	100 mL Whirl- Pak <sup>™</sup> bag	No additives	Keep fresh sample on wet ice (or refrigerated) and in the dark. Keep at 0- 4°C; do not freeze. Send so that sample is received within 2 weeks of sample collection

Analyte	Container	Preservation	Temperature & Holding Time
Diatom sample	50 mL centrifuge tube	Add 5% formalin for a 1% final concentration immediately after collection. Formalin does not need to be buffered, but if it is, use phosphate buffer, not borax. Samples should be fixed before shipment to lab. Note: glutaraldehyde is an acceptable alternative to formalin	Keep sample in dark and away from heat; fixed samples can be stored at 0-4°C for at least 2 years
DNA sample	Not applicable	Between 5-50 ml of algae composite sample is filtered onto a 0.45µm nitrocellulose filter. Filter is submerged in lysis buffer preservation solution and stored in microcentrifuge tube	Sample is kept in the dark and on ice until transfer to a -20°F (or -80°F if available) freezer. Fixed samples can be stored frozen for 2 years

### Table 2. Laboratory MQOs for Algal Taxonomic Analysis

Lab Quality Control	Frequency of Analysis	Measurement Quality Objective	Purpose
Similarity of diatom composition	10% of samples in project	Bray-Curtis similarity: $\sum min(QC_i, OR_i) \ge 70\%$ Where $QC_i$ is the percent abundance of taxon <i>i</i> reported by the QCT, and $OR_i$ is the percent abundance of taxon <i>i</i> reported by the ORT.	Precision, accuracy
Similarity of SBA composition	10% of samples in project	Sørensen similarity: $\frac{2 \times N_{COM}}{N_{OR} + N_{QC}} \times 100 \ge 80\%$ Where $N_{COM}$ is the number of taxa common for both taxonomists, $N_{OR}$ is the number of taxa reported by the ORT, and $N_{QC}$ is the number of taxa reported by the QCT. 80% similarity should be achieved individually for each SBA sample type (i.e. qualitative-macroalgae, quantitative – macroalgae, epiphytes, microalgae).	Precision, accuracy

Lab Quality Control	Frequency of Analysis	Measurement Quality Objective	Purpose
Photomicrograph agreement	SBA quantitative sample epiphytes and top 5 microalgal SBA taxa	At last 80% Sørensen similarity between ORT and QCT algae identifications based on review of the photomicrographs, submitted by ORT.	Accuracy, sensitivity
Sampling efficiency	100% of samples in a project	At least 600 diatom valves/300 SBA natural counting entities (NCE) are evaluated. If the sample is very sparse 300 diatom valves/150 SBA NCE are counted allowing 4 hours for sample analysis.	Completeness
Taxonomic Identification	100% of samples in a project	100% of all collected and sorted samples are processed.	Completeness
Sample homogenization	100% of samples in a project	SBA macroalgal clumps are manually extracted from the composite algae sample. Diatoms and SBA quantitative liquid fraction remaining after macroalgae removal are well homogenized prior to processing and analysis of microalgae fraction.	Representativeness

#### Table 3. Laboratory Quality Control Corrective Actions for Algae Analysis (SBA and diatoms)

Lab Quality Control	Corrective Action
Diatom Quantitative Sample Integrity Check	If sample is not preserved in formalin prior to receipt, it must be noted in the COC and the sample should be fixed immediately upon receipt. 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, and in comments section of AA taxa form. Results must be flagged if the hold time is missed. If a vial is cracked or leaking it must be transferred to a new vial according to the procedures outlined in Section 2.2.4 of Stancheva et al., 2015. If the sample is field-preserved but has a total volume less than 50 mL (40 mL sample and 10 mL formalin preservative), results must be flagged to indicate the reduced total sample volume.

Lab Quality Control	Corrective Action
Taxonomic Nomenclature	If taxonomic nomenclature is different from that in the SWAMP Master list, taxa names must undergo external taxonomic harmonization in order to be submitted as new records to the SWAMP Master List.
Photographic Documentation of Algae	If photomicrographs are absent for newly recorded species, the vouchered samples should be re- examined and photographs taken of the species in question by the taxonomist that recorded said species. If the quality standards are not met for the photomicrographs, multiple images should be collected until satisfactory image quality is achieved.
Standard Taxonomic Effort (STE)	(Coming soon) The STE provides guidance on the assignment of taxonomic identity for ambiguous taxa. This guidance includes the aggregation to genus level for morphospecies of certain routinely problematic genera.
External Taxonomic Harmonization (for SWAMP projects)	<ul> <li>Taxonomic identification of new records for SWAMP Master Lists should be approved by harmonizing taxonomist. The discussion between the OR taxonomist and the harmonizing taxonomist should continue until identification is settled.</li> <li>If additional photomicrographs need to be taken for newly recorded species, key aspects of vegetative and reproductive morphology should be documented and provided to harmonizing taxonomist.</li> <li>Voucher specimens should be sent upon request if photomicrographs are insufficient.</li> </ul>
Internal taxonomic QC	Taxonomic disagreement between both taxonomists should be resolved and SBA and diatom taxonomy and enumeration should be corrected as needed in all project samples affected. For instance, if a name is confirmed to be systematically misapplied, all the samples from the project should be revisited, and the name should be corrected by the ORT and verified by the QCT. This step should be done internally before reporting the results, which improves the quality of the data.
Soft-bodied algae quantitative sample processing - Macroalgal fraction separation check	If macroalgae or other solid particles are observed in any of the original 50 mL centrifuge tubes checked by the QCT after macroalgae processing, all samples from the project should be reexamined for potentially omitted macroalgae. All remaining macroalgae should be added to the tube with the macroalgal fraction, and the total volume of macroalgae should be corrected accordingly.
External taxonomic QC	All samples that fail the MQO thresholds must undergo taxonomy reconciliation. The reconciliation process is conducted by the QCT in dispute with the ORT. All problematic taxa listed in SBA/Diatom QC Summary Tab in SBA/Diatom QC Submittal Data Template should be resolved for all QC samples to pass the MQO thresholds. Discrepancies are clarified by comparing

Lab Quality Control	Corrective Action
	the photomicrographs of questionable taxa with existing literature and established SWAMP photomicrograph records available in the California Online Algae Identification Resource Tools website. Additional algal material from the same sample may be examined to correct identifications.
	In case the MQO failures are due to misidentifications by ORT of common and abundant taxa based on feedback from the QCT, the project coordinator should choose corrective action, consisting of additional external QC of 10% of samples, when the data for a certain project are finally generated by ORT. However, this corrective action is possible for quantitative diatom and SBA samples, but not for qualitative SBA samples.
	The project coordinator selects another random 10% of samples to submit for a second round of QC. Samples that underwent QC in Round 1 should not be selected for Round 2 and subsequent rounds. If an additional round of QC is needed, all steps in the process are performed again, including submittal of an Excel Algae QC Submittal Data Template with data from the second set of samples, except that round would equal 2. The process continues until the OR lab, QC lab and project coordinator agree the data meet QC requirements, discrepancies have been resolved and data are finalized. Enforcement of corrective actions is the responsibility of the project coordinator, not the QC lab.

## References

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# **Glossary of Acronyms and Terms**

Acronym/Term	Definition
AA Taxa Form	Taxonomy data template with sample/collection information included; used by the laboratory to complete analysis calculations and submit results to SWAMP
COC	Chain of Custody form
MQO	Measurement Quality Objectives
NCE	Natural Counting Entities
OR	Original (Laboratory or Taxonomist)
ORT	Taxonomist who performs the original taxonomy analysis
QC	Quality Control (Laboratory or Taxonomist)
QCT	Taxonomist who performs the taxonomy quality control
SBA	Soft-bodied algae
STE	Standard Taxonomic Effort document
SWAMP	Surface Water Ambient Monitoring Program (State Water Resources Control Board)
SWAMP Master list	SWAMP's Master List of SBA and diatom organism names