SWAMP
Surface Water
Ambient Monitoring
Program

SWAMP Bioassessment Procedures 2019

Standard Operating Procedures for Internal and External Quality Control of Laboratory Processing, Identification and Enumeration of Stream Algae in California

November 2019

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ACKNOWLEDGEMENTS

The current version of the protocol was developed with discussion contributions from the following people:

Christina Fuller and Kim Conklin (California State University, San Marcos) - technical assistance and discussion

Kalina Manoylov (Georgia College and State University) - SOP review and comments

John Wehr (Fordham University) - SOP review and comments

Betty Fetscher (San Diego Regional Water Quality Control Board) - SOP review and comments

Candice Levesque (State Water Resources Control Board) – preparing Appendix I and II

Peter Ode (Department of Fish and Wildlife Aquatic Bioassessment Laboratory)

Raphael Mazor (Southern California Coastal Water Research Project)

Andrew Rehn (Department of Fish and Wildlife Aquatic Bioassessment Laboratory)

Calving Yang (State Water Resources Control Board)

Jennifer Salisbury (State Water Resources Control Board)

Lori Webber (State Water Resources Control Board)

Marco Sigala (Moss Landing Marine Laboratories)

Melissa Morris (State Water Resources Control Board)

Tessa Fojut (State Water Resources Control Board)

Richard Mitchell (Monitoring Branch, U.S. EPA's Office of Water)

Sean Sullivan (Rhithron Associates, Inc.)

Isabelle Lavoie (Institut National de la Recherche Scientifique, Canada)

Citation for this document:

Stancheva, R. and Sheath, R. G., 2019. Standard Operating Procedures for Internal and External Quality Control of Laboratory Processing, Identification and Enumeration of Stream Algae in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP.

LIST OF ACRONYMS AND ABBREVIATIONS

Term	Definition
Δ IBI D18	Difference in IBI D18 scores
Algae Lab SOP	Standard Operating Procedures for Laboratory Processing, Identification and Enumeration of Stream Algae (Stancheva et al., 2015)
BCS	Bray Curtis Similarity
COC	Chain of Custody
DI water	Deionized Water
ID	Identification
MQOs	Measurement Quality Objectives
NCE	Natural Counting Entity
OR	Original Laboratory
ORT	Taxonomist who performs the original taxonomy analysis
QA	Quality Assurance
QC	Quality Control
QCT	Taxonomist who performs the taxonomy quality control
Sample ID	Unique sample name
SBA	Soft-bodied algae
SDT	Submittal Data Template
SOP	Standard Operating Procedures
SS	Sørensen Similarity
STE	Standard Taxonomic Effort
SWAMP	Surface Water Ambient Monitoring Program
SWAMP IQ	SWAMP Information Management and Quality Assurance Center

INTRODUCTION

This document describes the standard operating procedure (SOP) for internal and external laboratory quality control (QC) of soft-bodied algae (SBA) and diatom data generated for the Surface Water Ambient Monitoring Program (SWAMP) and participating SWAMP-comparable bioassessment projects in California. This document is to be used in conjunction with the Standard Operating Procedures for Laboratory Processing, Identification, and Enumeration of Stream Algae (Algae Lab SOP, Stancheva et al. 2015).

Internal lab QC occurs in the original laboratory (OR lab) where SBA and diatom samples are analyzed with the goal to support production of algal taxonomy data with high precision and quality by verifying the accuracy of: 1) SBA sample processing, and 2) taxonomic identification of SBA and taxonomic identification and enumeration of diatoms in accordance with established Standard Taxonomic Effort (STE, provided in Algae Lab SOP, Section 1.6). External QC occurs when SBA and diatom samples that have been processed and analyzed in the OR laboratory are sent to an independent external QC lab for confirmation of algal identification and enumeration. Taxonomist and technician qualifications and other laboratory practices required for quality control are the same as those described in the Algae Lab SOP, Section 1.

The goals of internal and external QC are: 1) to ensure that taxonomic identifications are in accordance with STE, and 2) to provide quantitative measurements of the accuracy and precision of taxonomic data that can be compared against established standards, known as measurement quality objectives (MQOs). The QC process promotes continuous real-time taxonomic training of all taxonomists to support taxonomic consistency of algae data produced by multiple labs and allows users to evaluate data quality.

SECTION 1: GENERAL CONSIDERATIONS IN ALGAE TAXONOMY QC

A cornerstone of taxonomic QC is that OR and QC taxonomists need to have a high likelihood of observing the same algal specimens. However, in algal bioassessment, it is not possible to separate the microscopic single-celled organisms in a container for taxonomy QC, nor to mark the exact area that was analyzed on the slide. Therefore, the possibility of incomplete overlap in algal specimens observed by the two taxonomists (i.e., influence of within-slide and within-sample taxonomic variability on similarity results) is considered in setting MQO thresholds. Furthermore, the holding time for the preserved SBA QC samples should be minimal, because their processing and analysis in the OR lab exposes algae to light, high temperature, air, and DI water, which compromise cell and pigment preservation. Typically, low volumes of the algal fractions analyzed (see below) and their transportation to the external QC lab may result in fragmentation of algal thalli, which prevents their species level identification by the QCT. QC procedures for each type of algal sample and some technical limitations are outlined below.

1.1 SBA QUALITATIVE SAMPLE

This sample is large (40-500 mL), unpreserved and perishable. As such, it must be stored at 4°C and analyzed fresh, as soon as possible after its collection. This means that the fresh qualitative sample cannot be transported to the external QC lab. Internal taxonomy QC is performed on the same fresh sample the same day. Then, a small representative composite subsample (10-40 mL), containing all macroalgal taxa, is preserved and vouchered (see Algae Lab SOP, Section 4.3.1). The vouchered preserved subsample is sent to the external QCT for macroalgae ID confirmation based on direct sample observation and review of algae photomicrographs provided by the ORT. The qualitative QC sample is washed for preservative removal by the QCT, which may cause damage and loss of algae. A smaller amount of algal material available to the QCT may limit the observation of rare species, represented with single filaments in the qualitative sample.

1.2 SBA Quantitative Sample - Macroalgal Fraction

Minimally disturbed and properly stored macroalgal fractions after the OR analysis ideally lead to observations of the same macroalgal specimens and algal epiphytes by the QCT for internal and external QC. The entire content of the macroalgal fraction (typically 0.1-0.5 mL) is theoretically available to the external QCT for ID confirmation of macroalgae and epiphytes based on direct sample observation and algae photomicrographs provided by the ORT. However, sample transportation and preservative removal may cause damage and loss of algae.

1.3 SBA Quantitative Sample - Microalgal Fraction

The microalgal fraction is small (1 mL) and the subsample analyzed by the ORT is not available to the external QCT, because one drop is viewed on a water semi-permanent mount, which cannot be stored for a long period and cannot be transported. This fraction undergoes internal QC on the same slide, i.e., is analyzed by both the ORT and internal QCT on the same day for verification of microalgae identification. For external QC, the five most abundant microalgal taxa are selected based on the number of natural counting entities (NCEs) recorded for each, and their species identification is confirmed by the QCT based on photomicrographs, descriptions and direct analysis of the remaining microalgal fraction. However, sample transportation and preservative removal may cause loss of algae.

Previous QC data showed that SBA samples degrade shortly after their initial processing, microscope identification, re-preservation and transportation to the extent that the QCT recorded a significantly lower number of algal species than the ORT. Therefore, the SBA taxonomy QC is based primarily on review of high-quality algal photomicrographs collected by the ORT, following the standards described in the Algae Lab SOP, Section 1.5. and Appendix H.

1.4 Diatom Sample

Permanent diatom slides with fixed diatom valves distributed within a known analyzed area are subject to internal and external QC for complete taxonomic ID and enumeration. However, shifts in the actual area counted by both taxonomists may exist due to differences in microscope stage micrometers and diameters of the field of view, even when the first counted field of view is indicated by etched lines on the cover slip and illustrated with low-magnification pictures (see

Section 4.2.1).

SWAMP requires external taxonomy QC of 10% of samples and internal taxonomy QC of 5% of samples when a second taxonomist is available in the OR lab. If a second taxonomist is not available, the internal QC is not performed. A summary of all types of algal samples subject to taxonomy QC analysis is presented in Table 1.

Table 1. Algae samples and data subject to internal and external taxonomy QC analyses. Legend: Measures used to define quality objectives (MQOs; see section 5.1): Sørensen similarity (SS), Bray-Curtis similarity (BCS), and Difference in IBI D18 scores (Δ IBI D18).

Sample Type	Data Subject to QC	Quality Objective	MQOs
SBA qualitative	Macroalgae taxa list	Taxonomic precision Completeness	SS
SBA quantitative – macroalgae	Macroalgae taxa list	Taxonomic precision Completeness	SS
SBA quantitative – epiphytes	Epiphytes taxa list	Taxonomic precision	SS
SBA quantitative – microalgae	Top 5 microalgae taxa list	Taxonomic precision	SS
Diatom	Diatom taxa list with diatom valves enumerated	Taxonomic precision Enumeration precision	BCS Δ IBI D18

SECTION 2: INTERNAL QC PROCEDURE FOR SBA QUANTITATIVE SAMPLE PROCESSING

Algal sample processing for taxonomic analysis has a large impact on the accuracy of taxonomic data for both diatoms and SBA. Internal laboratory QC of SBA quantitative sample processing is a feasible step that can improve data accuracy. SBA taxonomic analysis is based on separate processing of macroalgal and microalgal fractions of the quantitative sample. The separation step is performed typically by the OR laboratory technician, but could be done by the taxonomist as well. Complete separation of the macroalgal fraction from the original composite sample is a

crucial step because it significantly influences algal species identification and biovolume estimate. Therefore, SWAMP requires QC on the macroalgal fraction separation. For each project, 10% of processed SBA quantitative samples (i.e. samples in which the macroalgal and microalgal fractions have been separated) are randomly selected and checked by a taxonomist before proceeding to taxonomic analysis.

Step 1: Visually examine the remaining liquid in the original 50 mL centrifuge tube with the composite sample for the presence of any remaining macroalgae or other solid particles by gently but thoroughly inverting the centrifuge tube several times to see floating particles with the naked eye.

Step 2: Under a dissecting microscope, visually examine the remaining liquid in the original 50 mL centrifuge tube with the composite sample for the presence of any remaining macroalgae or other solid particles.

Corrective Action: If macroalgae or other solid particles are observed in any of the original 50 mL centrifuge tubes checked, all samples from the project must be reexamined for potentially omitted macroalgae. All remaining macroalgae are then added to the tube with the macroalgal fraction, and the total volume of macroalgae is corrected accordingly (see Algae Lab SOP, Section 3.1.2).

Recording Data: Record the results in an excel file named SBA Sample Processing QC Submittal Data Template (SDT). For each sample subjected to internal QC, include: Project Code, Station Code, Sample Date, Quantitative sample - macroalgae left in the original 50 mL tube (yes or no), QC Date, OR technician name, and QC technician name. The OR lab is responsible to keep the records from the internal QC of SBA sample processing.

SECTION 3: INTERNAL TAXONOMY QC FOR SBA AND DIATOM SAMPLES

Internal SBA taxonomy QC should be performed on the same day as the OR analysis immediately after the OR analysis is completed. In this way, both taxonomists view the same algal specimens. MQOs for internal SBA and diatom taxonomy QC are the same as for external taxonomy QC (see Section 5).

SBA algal samples for internal taxonomy QC include:

1. entire contents of the fresh qualitative SBA sample;
2. petri dish with entire content of minimally disturbed macroalgal fraction of SBA quantitative sample;
3. semi-permanent sealed water mounts with macroalgae used for species IDs by ORT; and
4. semi-permanent sealed water mount with marked counted area of microalgal SBA quantitative fraction.

Step 1: The internal QCT randomly selects 5% of SBA and diatom samples from each project for internal taxonomy QC.

Step 2: When the ORT finishes the sample analysis, the algal material is passed to the internal QCT for taxonomic analysis the same day. For QC SBA samples, the internal QC analysis follows the Algae Lab SOP, Sections 4.1.1 to 4.1.3; MQOs are calculated for the same parameters considered for the external QC. The diatom QC procedure should follow the steps described in Section 4 below.

Corrective Action: Taxonomic disagreement between the two taxonomists should be resolved and algal taxonomy and enumeration corrected as needed in all project samples affected. For instance, if a name is misapplied in samples, for which MQOs are failed, all samples from the project are revisited, and the name corrected by the ORT and verified by the QCT. This step should be done internally before reporting results to improve data quality.

Recording Data: Record the results from the internal taxonomy algae QC in the Excel SBA/Diatom QC SDT, following the same reporting instructions as for external QC. The OR lab is responsible to keep the records from the internal taxonomy QC.

SECTION 4: EXTERNAL TAXONOMY QC FOR SBA AND DIATOM SAMPLES

Three parties (e.g. project coordinator, ORT, and QCT) collaborate during external QC, as outlined below and illustrated in Appendices I and II. Random selection of QC samples is recommended in nearly all cases. It is critical that QC samples are not selected with bias; otherwise, QC results will not be representative of larger projects. However, in some occasions, the composition of algal samples could be inappropriate for taxonomic comparison and may not be representative for large data sets. Examples for SBA are: quantitative samples with very low taxa richness (e.g., <5 taxa) or missing macroalgae, or low abundance of microalgae (e.g., <150 NCEs). Also, if the selected QC qualitative SBA samples are in poor condition from improper storage or preservation, voucher specimens from that sample may no longer be identifiable and therefore not appropriate for QC. For diatom samples, if the sediment volume is high (typically above 10 mL in 50 mL tube), the sample is considered inappropriate for taxonomy QC. This is because large amounts of sand and fines observed in the diatom slide interfere with diatom identification.

The QC samples are the first samples to be analyzed from a certain project, because the holding time of the processed, analyzed and re-preserved SBA QC samples is short (maximum 6 months). Therefore, the SBA QC samples must be accompanied with detailed high-quality photomicrographic documentation of each identified algal taxon.

Step 1: The project coordinator randomly selects 10% of samples for taxonomy QC before field sampling, following Woodard et al. (2012).

Step 2: The project coordinator indicates the QC samples on the COC, which are sent to the OR lab along with the samples.

Step 3: The ORT checks the selected QC samples for their condition and content upon delivery to the OR lab. Visually check the vials with diatom QC samples for the level of sediment (unacceptable volume is typically above 10 mL in 50 mL tube), and SBA QC samples for the amount of algae (quantitative samples with very low taxa richness (e.g., <5 taxa) or missing macroalgae, or low abundance of microalgae (e.g., <150 NCEs) are inappropriate for QC).

Step 4: The OR lab sends the project coordinator a report describing the condition of algae QC samples after their microscope analysis, along with recommendation for replacement of inappropriate samples, if any. Discretion of the project coordinator is used in such cases to select alternate samples (Rehn et al., 2015).

Recording Data: The QC communication between labs is recorded in the Excel SBA/Diatom QC SDT. It is mandatory that in the QC SDT only SWAMP Project and Stations Codes are used (i.e., not internal lab codes), and that all algal names are obtained from the SWAMP Master Algae and Diatom Lists available at: http://swamp.waterboards.ca.gov/swamp_checker/DisplayLookUp.aspx?List=OrganismLookUp_Algae, and http://swamp.waterboards.ca.gov/swamp_checker/DisplayLookUp.aspx?List=OrganismLookUp_Diatom. The OR lab is responsible to keep records from the external taxonomy QC.

4.1 PROCEDURES FOR TAXONOMY QC OF SBA SAMPLES

4.1.1 Procedures for the OR Lab

Step 1: Analyze qualitative and quantitative SBA QC samples following the Algae Lab SOP. In addition, for the purpose of the QC process, take high-quality photomicrographs (follow Section 1.5. and Appendix H of the Algae Lab SOP) of each algal species observed in all sample types and fractions (see Table 1).

Step 2: When OR identification of the quantitative SBA QC samples is completed, retrieve the centrifuge tubes with macroalgal and microalgal fractions and add 3-5 drops of 2% glutaraldehyde to each tube to re-preserve for shipment. Retrieve the vial with qualitative vouchered sample belonging to the selected QC sample from the sample archive. Make sure that there is parafilm around the cap of each vial. Add the label “QC sample” to the tubes and vial.

Step 3: Retrieve ID Datasheets (Algae Lab SOP, Appendix C) for the selected QC samples. Retrieve all algal photomicrographs collected during identification of QC samples. This information is used in taxonomy QC analysis, review and reconciliation.

Step 4: Prepare the SBA QC COC and populate the following tabs in the SBA QC SDT:

- *Macroalgae QC Results Tab*, which contains a list with all SBA macroalgae identified in the qualitative sample and in the quantitative macroalgal fraction, and list with all SBA epiphytes recorded in the quantitative macroalgal fraction, along with names of photomicrographs of each algal ID.
- *Microalgae QC Results Tab*, which contains a list of the top five species in SBA quantitative microalgal fraction, including the number of NCEs, size ranges, and other key morphometric features, along with names of photomicrographs of each microalgal ID.

Step 5: Send to the QC lab the following materials:

- SBA QC samples;
- SBA QC COC, which contains a list with all SBA QC samples;
- SBA QC SDT with ORT data (see section 4.1.1 step 4);
- SBA photomicrographs of all SBA IDs (see section 4.1.1 step 1);

Reference Appendix I for a flowchart illustrating the general process outlined in this section.

4.1.2 Procedures for the QC Lab

Sample Receipt

Step 1: Upon receiving QC samples, the QCT checks all samples for their condition, confirms that they match the information in the SBA QC COC, and makes any relevant notes in the COC.

Step 2: The QCT verifies the SBA QC SDT contains all required ORT data.

Qualitative Sample

Step 1: Retrieve the plastic vial labeled “Qualitative sample”. Transfer the entire content of the vial into 50 mL tube by multiple rinsing with DI and add DI to the top of the tube. Wash the

sample carefully by soaking in DI water for at least 2 hours to remove the glutaraldehyde. Remove the supernatant carefully with a pipette without disturbing the algae.

Step 2: Gently shake the tube and pour the sample into a glass dish. Wash the tube with DI to remove any algae adhered to the walls and pour it into dish. Observe thoroughly and identify to species level all SBA macroalgae, following the steps outlined in Section 4.1.1, Algae Lab SOP.

Step 3: Review the ORT macroalgae taxa list and photomicrographs, and verify the taxa IDs. If there is disagreement with OR IDs, or if macroalgae absent in the OR list are observed in the sample, take high-quality photomicrographs (follow Section 1.5. and Appendix H in the Algae Lab SOP) of the taxa in question.

Step 4: Record all identified SBA macroalgal taxa along with the corresponding photomicrographs in the *Macroalgae QC Results Tab* in SBA QC SDT. Provide confirmation for ORT macroalgal identifications and photomicrographic documentations.

Step 5: Return the macroalgal material to the vial and add DI water and glutaraldehyde to a 2% final concentration.

Quantitative Sample - Macroalgal Fraction

Step 1: Retrieve the 15 mL or 50 mL centrifuge tube with the macroalgal fraction.

Step 2: Add DI water to the top of the tube to dilute the glutaraldehyde. Settle the sample overnight, and the next day, very carefully remove the water with a pipette, ensuring that the algae are not disturbed or lost.

Step 3: Gently shake the tube and pour the sample into a glass dish, identify each SBA macroalgal and SBA epiphytic taxon following the steps in Section 4.1.2, Algae Lab SOP, excluding algae quantification.

Step 4: Review the ORT macroalgae and epiphyte list and photomicrographs. If there is a disagreement with OR IDs, or macroalgae absent in the OR list are observed, take high-quality photomicrographs (follow Section 1.5. and Appendix H of the Algae Lab SOP) of the taxa in question.

Step 5: Record all identified SBA macroalgal taxa along with corresponding photomicrographs in the *Macroalgae QC Results Tab* in SBA QC SDT. Provide confirmation for macroalgal taxa and epiphytes identified and illustrated by the ORT.

Step 6: Return the macroalgal material to the vial and add glutaraldehyde to a 2% final concentration.

Quantitative Sample - Microalgal Fraction

Step 1: Review the pictures and descriptions of the five most abundant microalgal species and verify species identifications.

Step 2: Retrieve 15 mL centrifuge tube with microalgal fraction and prepare microscope water mount following Section 3.1.4 in the Algae Lab SOP.

Step 3: Scan the slide at 20x to locate the taxa of interest.

Step 4: Identify the most abundant microalgal species at 40x. If there is a disagreement with OR IDs take high-quality photomicrographs (follow Section 1.5. and Appendix H of the Algae Lab SOP) of the taxa in question.

Step 5: Provide confirmation for the top five microalga taxa identified and illustrated by the ORT in the *Microalgae QC Results Tab* in SBA QC SDT.

Step 6: Discard the analyzed slide properly.

4.2 PROCEDURES FOR TAXONOMY QC OF DIATOM SAMPLES

4.2.1 Procedures for the OR Lab

Additional Materials Needed:

- High precision diamond scriber (Thomas Scientific, Swedesboro, NJ)

Step 1: Retrieve the permanent diatom slide belonging to the selected QC sample, which is already analyzed and labeled according to Algae Lab SOP, Sections 4.2 and 4.3.3. Verify that each slide has etched line indicating the first transect analyzed and clearly denoted start point for each transect counted, recorded with coordinates from the microscope stage (Algae Lab SOP,

Section 4.2 Step 4, Appendix C2). High precision diamond scriber (Thomas Scientific, Swedesboro, NJ) may be used for etching lines on the surface of the cover slip. It is important that all microscope stage coordinates should be recorded from a slide positioned with its label to the right. The first field of view should be indicated additionally as illustrated in Figure 1 by:

- cross section of horizontal and vertical lines etched on the surface of the coverslip;
- black dot with permanent marker outside the cover slip next to the first field of view;
- medium-magnification (40x) images of the first field of view.

Step 2: Retrieve ID Datasheet (Algae Lab SOP, Appendix C2) and all diatom photomicrographs collected during the analysis of the samples selected for QC to support taxonomy QC review and reconciliation.

Step 3: Prepare Diatom QC COC and populate the following tabs in the Diatom QC SDT:

- *Diatoms QC Results Tab*, which contains the diatom species list with corresponding valve enumeration data.

Step 4: Send to the QC lab the following materials:

- Permanent diatom QC slides;
- Diatom QC COC which contains a list of QC samples, image names of the first fields of view along with information from Appendix C2 in the Algae Lab SOP;
- Diatom QC SDT with the OR taxonomy data;
- Electronic images of the first fields of view.

Reference Appendix I for a flowchart illustrating the general process outlined in this section.

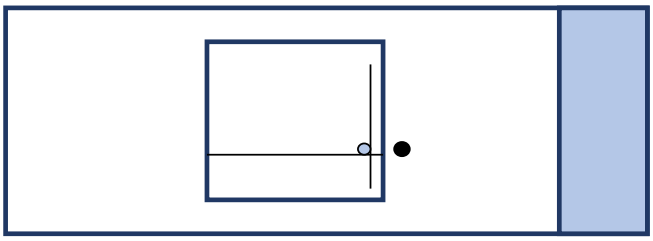

Figure 1. Diagram of diatom microscope slide positioned with its label to the right, showing the crossing etched lines on the cover slip to indicate the first field of view, which is the blue circle.

4.2.2 Procedures for the QC Lab

Step 1: Verify that all slides and images received match the information in the COC. The OR data should be scanned for completeness, slide conditions and if problems are observed they should be recorded by the QC lab in the Diatom QC COC.

Step 2: Position the slide on the microscope stage with its label to the right. Take the coordinates of the upper right corner of the cover slip and recalculate the coordinates of the first and last field of view for each counted transect by the OR taxonomist.

Step 3: Find the first counted field of view using the stage coordinates and with the help of the crossed etched lines on the cover slip. Position the center of the ocular micrometer at 40x according to the coordinates. Locate the crossed etched lines on the cover slip (see Figure 1) and identify the first field of view, which is viewed below the horizontal line. The top of the first field of view should touch the etched horizontal line and the left side should touch the vertical line. Check the picture from the OR to confirm that you see the same diatom valves. If not, adjust the slide accordingly. Continue counting to the right along the horizontal transect started with the first field of view.

Step 4: Identify and enumerate all diatom taxa following the steps in Section 4.2 from Algae Lab SOP.

Step 5: Take photomicrographs of diatom taxa in question, because they are used in discussion with the OR taxonomist.

Step 6: Record the QC results in the *Diatom QC Results Tab* in the Diatom QC SDT and match the taxa IDs common for both taxonomists along with counted number of valves.
Reference Appendix II for a flowchart illustrating the general process outlined in this section.

SECTION 5: ANALYSIS OF TAXONOMY QC RESULTS, DATA RECONCILIATION AND CORRECTIVE ACTIONS

Measurement quality objectives (MQOs) are established standards for evaluation of the accuracy of taxonomic identification and enumeration of algal data during the QC process. These calculations are a powerful tool that laboratories may use to assess QC and to discover and address QC issues that may otherwise go undetected. However, in algal bioassessment the values of the MQOs are affected by variable subsampling errors for different types of samples. Results of the QC check should be analyzed to verify that identification and enumeration results meet SWAMP QA standards to determine data usability. If MQOs are not met, corrective actions must be carried out.

5.1 MQO THRESHOLDS

5.1.1 MQO Thresholds for SBA

The aim of SBA QC is validation of the taxonomic precision of all ORT SBA identifications in accordance with STE, based on review of algae photomicrographic documentation and direct re-identification of the samples by QCT. SBA quantification precision for each taxon cannot be verified due to sample degradation over multiple treatments and transportation. The Algae Lab SOP requires all macroalgal species to be identified and recorded while microalgal identifications are limited to the first 300 NCEs observed and epiphytes are identified from random macroalgal portions. The same macroalgal fraction from each sample is available to both the ORT and the QCT; therefore, the taxonomic completeness of macroalgal composition in qualitative samples and macroalgal fraction of quantitative samples is verified and any macroalgal taxa omitted by the ORT are reported. In contrast, the ORT and the QCT analyze two different portions from the microalgal fraction and epiphytes with variable content of microscopic algal cells. Thus, the QCT only confirms the ORT identification precision for the top 5 microalgal taxa and epiphytes reported, based on review of algae photomicrographic documentation and direct re-identification of the sample. No microalgal and epiphytic taxa omitted by the ORT are reported, because different subsamples are observed by the taxonomists.

The SBA identification precision for a given sample is measured by Sørensen similarity (SS, Sørensen, 1948), comparing the total number of SBA taxa IDs reported by the ORT and the QCT. The MQOs and the thresholds for internal and external SBA QC are the same.

Sørensen similarity measures the inter-taxonomist variability and taxonomic precision for SBA for a certain sample using the following formula:

$$SS = 2\ N_{COM} / (N_{OR}+N_{QC})\ x\ 100$$

Where:

N_{COM} = number of taxa common for both taxonomists;

N_{OR} = total number of taxa reported by the ORT;

N_{QC} = total number of taxa reported by the QCT.

SS ranges from 0% to 100% (identical SBA taxa recorded). The SS threshold is $\geq$ 80%, and should be achieved individually for each SBA sample type, i.e., qualitative - macroalgae; quantitative - macroalgae; quantitative – epiphytes; quantitative - microalgae as indicated in Table 1.

5.1.2 MQO Thresholds for Diatoms

The MQOs and thresholds for diatoms are adopted from the National Rivers and Streams Assessment Program (NRSA) of the USEPA (2012, 2013) and from the Canadian diatom bioassessment QA/QC program (Lavoie and Campeau, 2016). Taxonomic similarity between two diatom samples is measured by Bray-Curtis similarity (BCS, Bray and Curtis, 1957), and is sometimes called “Percent Community Similarity” (Stevenson and Bahls, 1999) or percent taxonomic disagreement (USEPA, 2013). In addition, the difference in diatom index of biotic integrity (IBI) D18 score (Fetscher et al., 2014), obtained from ORT and QCT data is calculated for each sample. Small differences in diatom species composition, which do not lower BCS significantly, could affect IBI scores if the species involved are indicators of specific conditions used in IBI metrics (Lavoie and Campeau, 2016). By contrast, samples with low BCS between both taxonomists may produce identical or similar IBI scores if the species composition discrepancy is not a result of taxonomic error, but other factors such as problematic valve density and distribution, a high percentage of sand and silt, or within-slide variability when different

slide areas are counted. Previous diatom QC results showed that BCS does not correlate with differences in IBI D18 scores, in accordance with the conclusions from the Canadian diatom bioassessment QA/QC program (Lavoie and Campeau, 2016). The MQOs and thresholds for internal and external diatom QC are the same (Table 2).

Bray-Curtis similarity measures inter-taxonomist variability and taxonomic precision for diatom identification for a given sample using the following formula:

$$BCS = \Sigma\, Qi$$

Where:
Qi = the smaller of the two percentages (i.e. provided by the ORT and the QCT) of species i.
Sum across all species identified by both taxonomists.

BCS ranges from 0% to 100% (i.e. identical assemblages). The USEPA (2013) adopted a BCS threshold of $\geq$75% based on a benthic macroinvertebrate threshold value proposed by Stribling et al. (2008). However, the USEPA (2013) QC process is applied to diatom slides with a more precisely marked counted area compared to this protocol, i.e. by a diamond objective marker mounted on the objective turret (USEPA 2012) to assure that both taxonomists are analyzing the same diatom specimens. Recent BCS threshold validation studies (Lavoie and Campeau, 2016, and references therein) show that diatom assemblages with BCS $\geq$ 60% are similar enough to be regarded as replicate samples in diatom bioassessment programs in Canada and Europe, but for assemblages with low species diversity, BCS should be $\geq$ 70%. Diatom QC data in these validation studies were produced by analysis of different slide areas by both taxonomists, and therefore the BCS thresholds incorporate within-slide variability. In the current SOP, the manual marking of the counted transects does not guarantee that both taxonomists are analyzing the same specimens, especially when more than one transect is scanned. Therefore, a BCS threshold $\geq$ 70% is acceptable considering the influence of within-slide variability on similarity results.

Difference in IBI D18 scores (Δ IBI D18) measures the consistency among analytical bioassessment results obtained for a given sample identified by different taxonomists. IBI D18 is calculated using a calculator for southern California algal Indices of Biotic Integrity (SCCWRP, 2014).

$$\Delta \text{ IBI D18} = \left| \text{IBI D18}_{OR} - \text{IBI D18}_{QC} \right|$$

Where:

IBI $D18_{OR}$ =IBI D18 score for a given sample calculated from ORT data;

IBI $D18_{QC}$ =IBI D18 score for a given sample calculated from QCT data.

ΔIBI D18 ranges from 0 (identical scores) to 100 (completely different scores). Lavoie and Campeau (2016) analyzed the score deviation of a Canadian diatom biological index, which is on 0-to-100 scale (Lavoie et al., 2006), obtained by two taxonomists counting the same diatom slides. Authors concluded that a difference of 2 index score units for a given sample analyzed by two taxonomists corresponds to within-slide variability, and overall deviation of up to 7 index score units is satisfactory. It should be considered that the taxonomic foundations of IBI D18 and the Canadian biological index are different in terms of the total number of indicator taxa incorporated and the level of taxonomic resolution. Despite the differences between the indices, pilot diatom QC SWAMP 2016 results showed similar deviation in IBI D18 scores as in Canadian results (i.e. 0 to 8); therefore, a Δ IBI D18 threshold $\leq$ 7 is acceptable.

Table 2: MQO thresholds for SBA and diatom taxonomy QC.

Legend: Sørensen similarity (SS), Bray-Curtis similarity (BCS), Difference in IBI D18 scores (Δ IBI D18).

Sample Type	SS	BCS	Δ IBI D18
SBA	$\geq$ 80%	N/A	N/A
Diatoms	N/A	$\geq$ 70%	$\leq$ 7

5.2 External Taxonomy QC Summary, Reconciliation, Workshop, Data Updates, Corrective Actions and Data Storage

5.2.1 Taxonomy QC Summary and MQOs Calculation

SBA

Complete ORT data, i.e., species IDs and photomicrographs are provided to the QCT at the beginning of the QC process. Once QC identifications are completed, the QCT prepares two documents: 1) SBA Macroalgae and Microalgae QC Results Tabs in SBA QC SDT, where each ORT ID is either confirmed or rejected, and 2) SBA QC Summary Tab in SBA QC SDT, where

MQOs are calculated and all species for which discrepancies are recorded, including any macroalgae omitted by the ORT, are provided.

Diatoms

ORT data are available to the QCT, and when the external QC analysis is completed, the QCT prepares two documents: 1) Diatom QC Results Tab in Diatom QC SDT, where ORT and QCT species IDs and quantities are matched for all samples, and 2) Diatom QC Summary Tab in Diatom QC SDT, where MQOs and all species with taxonomic discrepancies between both taxonomists are provided. All diatom taxa identified by one taxonomist with relative abundance $\geq$ 1.5%, but not recorded by the other taxonomist, should be discussed, because they could be potentially misidentified. All common diatom taxa for both taxonomists, quantified with $\geq$ 3% difference in relative abundance should be discussed, because there might be quantification problems.

5.2.2 Pass/Fail Determination

If the project requires $\leq$ 10 QC samples, failure of any one or more assessment MQO in a single sample triggers corrective action. For projects with more than 10 QC samples, if a tenth of the samples fail the MQOs (i.e., 2 in 20 QC samples, 3 in 30 QC samples) corrective action is required.

5.2.3 Taxonomy Reconciliation and Workshop

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 SDT should be resolved for all QC samples to pass the MQO thresholds. Discrepancies are clarified by comparing 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.

All remaining problematic taxa indicated by the QC analysis are discussed further during the annual taxonomy workshop, organized by the California Primary Algae Laboratory. Discussions between the taxonomists are essential to maintain taxonomic consistency and achieve agreement

about species concepts and algal quantification. The aim of the workshop is to provide real-time training to all taxonomists, who continue their taxonomic communication during sample analysis from the same projects in order to create consistent taxonomic data with high precision. Taxonomic discussion among taxonomists helps with the understanding of the specific difficulties with some taxa, for instance that published knowledge of a taxonomic group is incomplete, or competing taxonomic concepts exists, thereby causing taxonomic discrepancies, and repeated failures. For example, cryptic or undescribed species may cause difficulty or ambiguity. The workshop discussions and decisions about the taxonomic treatment of problematic taxa is summarized in a report, to be used by all taxonomists in their work as a taxonomic reference to support consistency among labs. This information helps to inform future adjustments to the algae STE(s).

5.2.4 Data Updates and Corrective Actions

Once taxonomic reconciliation is completed, both OR and QC initial data are corrected and updated accordingly in the SBA/Diatom QC Reconciliation Tab in the SBA/Diatom QC SDT and MQOs are recalculated by the QCT.

If taxonomic error in the identification, enumeration, or recording of some taxa is discovered for the ORT, all samples analyzed by the ORT are to be re-analyzed and algal taxonomy updated when needed. All taxonomic updates resulting from taxonomy reconciliation in OR data should be reported to SWAMP.

For the final data report to SWAMP the updated taxonomy data after the reconciliation are used and the code “BQC” (denoting “taxonomy external quality control” in the SWAMP database) should be placed in the QACode column of the reporting template for all records of the samples subjected to external QC.

If the MQO failures are due to obvious misidentifications by the ORT of well described common taxa based on feedback from the QC lab, the project manager may choose corrective action, consisting of additional external QC of another 10% of samples.

The project manager 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 SBA/Diatom QC SDT 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 manager agree the data meet QC requirements, discrepancies have been resolved and data are finalized. Enforcement of the second round of QC is the responsibility of the project manager, not the QC lab.

5.2.6 Storage of QC Data and Metadata

Once the SBA/Diatom QC SDT is populated by the OR and QC labs and the QC process is complete (e.g., lab reconciliation and corrective actions), the SBA/Diatom QC SDT is submitted to SWAMP by emailing the file to the SWAMP IQ representative.

All data from the QC process, including the SBA/Diatom QC SDT, the taxonomy workshop report, all algal photomicrographs and notes exchanged between taxonomists should be kept in the OR lab and used as taxonomic reference.

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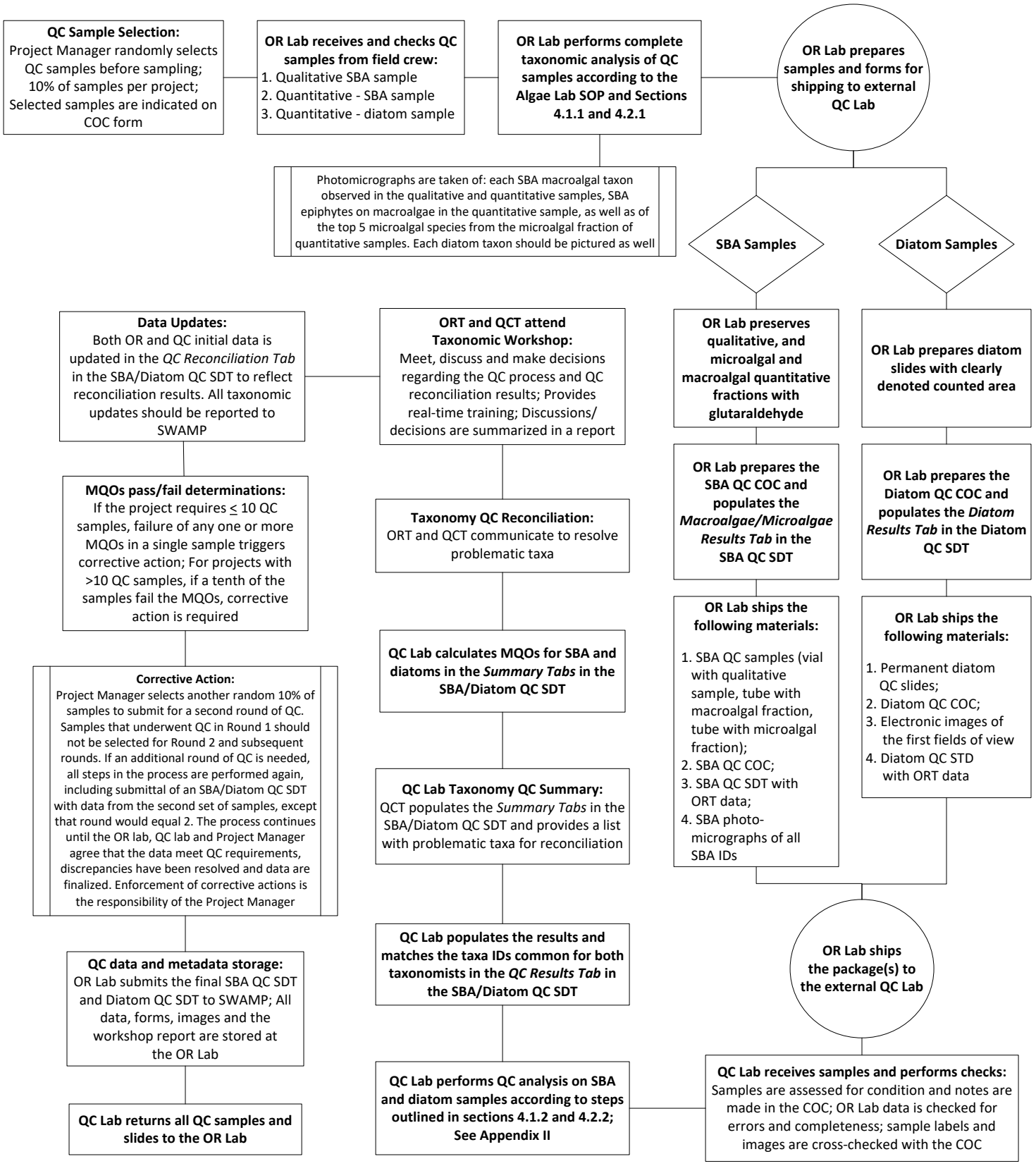
Appendix I: External Algae Taxonomy Process Flowchart



Appendix II: External Taxonomy QC Procedures Completed by the QC Laboratory