Algal Identification Quality Control (QC) Procedures: Initial Demonstration of Proficiency, Ongoing QC, Monthly QC, and Quarterly QC

1. SCOPE AND APPLICATION

1.1 This SOP outlines procedures for quality control for diatom and wet algal identifications in four parts: Initial Demonstration of Proficiency; Ongoing QC; Monthly QC; and Quarterly QC.

2. SUMMARY OF METHOD

2.1 For the initial demonstration of proficiency (IDOP), new analysts successfully pass QC (>70% similarity of taxa lists) for 10 samples. Another analyst then conducts ongoing QC on 10% of their samples until they have successfully passed (>70% similarity of taxa lists) 10 ongoing QC samples. After the IDOP, ongoing QC is conducted on 5% of samples.

2.2 Analysts participate in a Monthly QC exercise, which serves as ongoing training and a means of ensuring that analysts are making comparable identifications.

2.3 Analysts participate in Quarterly QC, for which they each analyze 3 samples twice and taxa lists are compared to determine intra-sample and inter-analyst variability (goal is >70% similarity between lists).

2.4 If any QC measure fails to pass, the source of the error(s) are determined and corrected. Further training may be conducted or samples may be re-analyzed.

3. APPARATUS AND EQUIPMENT

3.1 Primer-E software and documentation
Primer software and documentation may be obtained from Lab Data Support with Supervisor approval, provided there is sufficient license. This SOP is written for Version 5, but includes instructions for Version 6.

3.2 Microsoft Excel software

3.3 SBIO database

3.4 FDEP Laboratory Information Management System (LIMS)

3.5 Blank Algal identification bench sheet (printed from LIMS using the LIMS number assigned to the sample)

3.6 Phytoplankton and Periphyton Algal Identification QC Log (Analyst-specific, stored in B227A)

3.7 Algal sample(s)

3.8 Original algal identification bench sheet
The original bench sheet may be in one of several places depending on the stage of analysis or data entry in which the sample resides. Check with supervisor.

3.9 Initial Demonstration of Proficiency
3.9.1 Items 3.1 through 3.8
3.9.2 Training QC summary workbook (Analyst-specific folder with Excel files of all ID analyses and a summary)
\\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets

3.10 Ongoing Quality Control
3.10.1 Items 3.1 through 3.8
3.10.2 Working QC summary workbook (Wet or Diatom)
\\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets

3.11 Monthly Quality Control

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3.11.1 Items 3.2 and 3.7
3.11.2 Monthly QC Bench Sheet
\\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\Monthly_QC_Reports
3.11.3 Monthly QC Taxa List
\\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\Monthly_QC_Reports

3.12 Quarterly QC Procedures
3.12.1 Items 3.1 through 3.3 and 3.7
3.12.2 Quarterly QC Bench Sheets
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4. QUALITY CONTROL METHODS

4.1 Initial Demonstration of Proficiency (IDOP)

4.1.1 After a new analyst has read and signed off on all Algal Biology SOP's and the person(s) training the new employee feels that the new analyst has spent adequate time examining previously identified samples in conjunction with the bench sheets and has shown a willingness to ask for help with identifications, the new employee should re-identify a minimum of 10 samples previously identified by a proficient analyst. It is customary for new analysts to be trained on periphyton before phytoplankton.

Note: The new analyst's supervisor or designated trainer will choose samples to be re-identified. Samples to be re-identified for wet identification should have less than about 40% diatoms in the original ID. Samples to be re-identified for wet identification or diatom identification should not be samples with very low densities or taxa counts in the original ID or samples that were "problem" samples in the original ID such as very dirty samples. These samples are difficult to achieve 70% agreement on due to factors other than analyst proficiency, so using them as proficiency samples "stacks the deck" against the new analyst.

4.1.2 The new analyst then re-identifies the chosen samples. Wet identification should be performed using SOP AB-03 for periphyton and SOP AB-05 for phytoplankton. Diatom identification should be performed using SOP AB-03.1 or AB-05.1.

Note: If the sample is a phytoplankton or periphyton wet ID, the second analyst will prepare and settle a new aliquot of the sample, using the same dilution as the first analyst. If possible, a counting chamber with the same cell depth as that of the first analyst is used.

Note: If the sample is a diatom sample, use the permanent slide mount.

4.1.3 For each sample, create an Excel spreadsheet with the taxonomic identifications and unit counts, either by typing the names and units or by an SBIO retrieval (see note).

Enter the taxa names into the first column with the header "Taxa." Enter the original unit counts into the second column with the header "Count A." Count A is the person being QC'ed. Enter the second set of QC unit counts into a third column with the header "Count B." Enter zeros where there is no count. Insert a row at the top of the spreadsheet and type the file name into the first cell of that row (A1). Save the spreadsheet to the appropriate folder using the naming format of Trainee Analyst's Initials_Original Analyst's Initials_Quantitative/Qualitative_ Phytoplankton/Periphyton_LIMS#_analysis date.

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Note: To assist data entry, the taxonomic data for each sample can be retrieved from SBIO (taxa as entered, by sample, matrix, with phylogeny for the particular algal type), saved using the LIMS# as the file name, and opened in Excel. This provides most of the taxa and counts along with the phylogeny that can be used to create an aggregation file (step 4.1.4). Some taxa names, unit counts, and phylogeny may need to be added to the SBIO list. Save the phylogeny as a separate file "_Agg" before the .xls extension or as worksheet within the file.

Note: Save diatom ID QC spreadsheets to \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\QC-diatom training. Save wet ID QC spreadsheets to \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\QC-wet training.

Note: For example, a file for an initial proficiency re-identification performed by Jane A. Doe on a sample originally identified by John B. Deer on sample #123456, a qualitative phytoplankton sample, on March 17, 2008 would be named JAD_JBD_QL_PKD_123456_031708.xls.

Note: If a wet identification sample has cell counts, enter those into a separate spreadsheet. These counts will be used to assess whether or not cells are being counted correctly but will not be used for purposes of assessing identification proficiency.

4.1.4 Each QC sample should be analyzed separately. PRIMER-E (per SOP AB-15) will be used to conduct Bray-Curtis similarity analyses on these data, first with species level, non-transformed data. If the Bray-Curtis similarity is >70%, the QC analysis has been completed satisfactorily (passed). If the similarity value is <70%, repeat the analysis with a square root transformation. If the similarity is still < 70%, repeat the analysis at genus-level using a taxa aggregation file. If the similarity is still < 70%, conduct the genus-level analysis with square root transformed data. Record all similarity values, whether the QC passed at species or genus level, and the file name (.xls or _Agg.xls) on the QC bench sheet (paper), in the Excel taxa/unit file, and in the Excel QC Summary file.

Note: Square root transformation lessens the effect of differences in counts (large numbers of Alga #1 in sample A vs. B) when there are large differences in the number of units among the taxa present.

4.1.5 If the QC has passed at the generic level but not at the species level, compare the taxa lists from the two analysts. If differences exist between taxa lists, give additional training to bring the analyst up to the same level of proficiency.

4.1.6 If the QC does not pass at any level of comparison (genus vs. species) or at any level of transformation, perform the SIMPER analysis in PRIMER (per SOP AB-15) to determine which taxa contributed the most to the differences in counts.

Note: The SIMPER output lists the taxa in order of the greatest differences between the two counts. The Bray-Curtis dissimilarity between counts is one minus the Bray-Curtis similarity between counts. Each taxon that is not identical in the two samples contributes a portion of the inter-count dissimilarity.

4.1.7 Using the taxa list and Average Dissimilarity values generated from the SIMPER analysis, compare the differences between the taxa lists. Those taxa contributing the highest percentage

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of the dissimilarity between samples (from the column labeled "Contrib%" on the Results Log) should be examined to determine the source of the discrepancy (count, level of identification, etc.). All taxa contributing 5% or more of the total inter-sample dissimilarity should be examined.

4.1.8 The SIMPER results should be shown to the new analyst. The trainer and the new analyst should discuss any questionable identifications or counts while examining an aliquot of the sample in question.

Note: Sometimes what appears to be a counting error may simply be due to the patchiness of the sample distribution (i.e. clumps of algae that are not broken up during sample processing). This can usually be determined by looking at more of the sample.

4.1.9 If the QC does not pass at any level of comparison or transformation, the sample cannot be counted towards the ten successful re-identifications needed to establish proficiency.

4.1.10 Continue with re-identifications and analysis (steps 4.1.2 through 4.1.7) until 10 samples have been successfully re-identified.

4.1.10 After 10 samples have been successfully re-identified, the analyst in training should have 10% of samples QC'd by another analyst until 10 additional samples have passed the 70% QC threshold.

Note: After the second 10 samples have passed QC, the standard 5% of samples will be QC'd (see section 4.2 Ongoing Quality Control below).

4.1.11 Record the date of IDOP for the assigned Method in the LIMS training module.

4.2 Ongoing Quality Control

4.2.1 Each taxonomist records in his/her assigned Phytoplankton and Periphyton Algal Identification QC Log (Wet or Diatom) all the samples he/she has analyzed.

4.2.2 When 20 samples of a particular type have been processed, another analyst, who also performs that type of identification, is asked to randomly choose a sample from the list.

Note: For instance, if identifying diatoms, ask another analyst who does diatom identifications to perform the QC on that sample. If only one person performs that type of identification, s/he must QC his/her own sample.

4.2.3 The second analyst then identifies the selected sample following the appropriate SOP from step 4.1.2 and enters the data into an Excel spreadsheet following step 4.1.3. Save the spreadsheet to the appropriate folder using the naming format of First Analyst's Initials_Second Analyst's Initials_Quantitative or Qualitative_ Phytoplankton or Periphyton_LIMS#_analysis date.

Note: Save diatom ID QC spreadsheets to \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\QC-diatom working. Save wet ID QC spreadsheets to \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\QC-wet working.

Note: For example, a file for a QC performed by Jane A. Doe and John B. Deer on sample #123456, a qualitative phytoplankton sample, on March 17, 2008 would be named JAD_JBD_QL_PKD_123456_031708.xls.

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4.2.4 PRIMER-E will be used to conduct QC analysis and record similarity values per steps 4.1.4 and 4.1.7.

4.2.5 Identifications determined to be incorrect are corrected on the benchsheet and in SBIO. If the supervisor determines that the sample should be re-identified by both analysts, the re-identifications of any QC sample should be put through the steps outlined in this SOP to determine if the re-identification passed QC standards and if problem areas have been corrected. If a sample is re-analyzed and the second set of analyses passes the QC, the second result should be reported in SBIO.

Note: Within a sample, low algal density or high taxa evenness are two examples of why similarity may not meet the 70% threshold.

Note: If the supervisor and analysts determine that the differences in the taxa lists are due to variability within the sample or level of experience rather than identifications, the sample does not need to be re-identified.

4.3 Monthly Quality Control Exercises

4.3.1 Once a month, all analysts doing a particular type of identification participate in an internal Monthly QC exercise. The sample chosen for this monthly exercise should be one that has not been analyzed previously.

Note: The group can scan the sample to ensure that it has enough taxa to complete the exercise. Rotate samples chosen for monthly QC discussions through samples from recently collected periphyton (qualitative and quantitative) and phytoplankton. More than one sample can be used for each Monthly QC.

4.3.2 Prepare one aliquot or permanent slide mount for analysis following SOP AB-03 for periphyton prep, SOP AB-02.01 for diatom prep, or SOP AB-05 for phytoplankton prep.

4.3.3 Sitting around a single microscope, the analysts take turns choosing a specimen to identify. Without discussion, each analyst writes down his or her identification of the chosen specimen in the first column on the Algal Identification Monthly QC Bench Sheet, identifying the specimen to the lowest level of which s/he is capable.

Note: Using a single microscope, instead of each analyst using their own, removes variability associated with differences in scopes and gives all analysts the same view of each taxon.

4.3.4 After everyone has identified the specimen, the analysts compare identifications and discuss any differences.

Note: Monthly QC sessions are an opportunity for all analysts to share their expertise with each other. There is no pass or fail.

4.3.5 Once a consensus is reached, the “correct” identification is noted on the bench sheet in the second column. In cases where an agreement cannot be reached, the subsection supervisor will make a decision based on research.

Note: “Correct” is the best identification as agreed upon by the group. In some cases, it may be agreed that the most correct answer is to back up to a higher taxonomic level such as genus or family.

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4.3.6 If a consensus cannot be reached, the analysts will note the lowest level at which they agree as the "correct" identification. These taxa are tracked so that those, for which this occurs frequently, can be submitted to outside experts for resolution.

4.3.7 This exercise continues until 25 different taxa have been encountered or time runs out.

Note: In some cases the identifications and discussion take considerable time, especially with three or more analysts. It is not desirable to take more than one 8-hour period per month to complete this exercise. If possible, 25 different taxa should be identified.

4.3.8 Any incorrect identifications should be re-examined to identify the source of the problem. Adjustments, further training and/or outside consultation may be required.

Note: For instance, compare microscopes and adjustments to assure that all equipment is working properly. If analysts' microscopes differ, then their id's may also.

Note: Level of identification (genus or species) as a source of misidentification should be rectified with additional training, or with standardization of level of identification, as appropriate.

4.3.9 A cumulative list of taxa identified in Monthly QC exercises will be kept. A combination of new and previously identified taxa should be included in each Monthly QC session, if possible.

Note: The purpose of keeping track of taxa examined is to ensure that a wide range of taxa are used in Monthly QC sessions over time. This list is located in AlgalCommon /QCSpreadsheets/Monthly_QC_Reports.

4.4 Quarterly QC Procedures

The Quarterly Exercise is designed to assess all variability (intra-analyst, inter-analyst, intra-sample, and inter-sample). The analysis of this exercise may help identify potential areas of bias within taxonomic data generated by the Algal taxonomy Workgroup.

Detailed instructions are designed to apply to QC exercises with three analysts for either diatoms or soft algae. If only two analysts participate, four samples must be analyzed as described. If four analysts participate, only two samples need to be analyzed. This design gives the desired 16-18 data sets for statistical comparison, for which number of analysts is unimportant.

4.4.1 The Taxonomy Supervisor should choose and code three samples from the backlog for analysis. Samples for diatom and soft algae analysis should be of the same general type (phytoplankton, periphyton, Everglades, etc.), but different samples may be used. Once per year, the Taxonomy Supervisor will choose two samples rather than three. One sample will be analyzed as is. The other sample will be split into two subsamples. These subsamples will be analyzed as two separate samples. The supervisor setting up the exercise should retain a list with the identity of the samples. Analysts will not be told the identity of any sample analyzed in this exercise until the exercise is completed. They will be told the type of sample (periphyton, phytoplankton, etc.) as this determines how the sample is prepped and analyzed.

Note: Diatom samples should be chosen from those with high diatom abundance; soft algae samples should be chosen from those with high soft algal abundance. This can be determined with a preliminary scan of some sample material using the inverted microscope.

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4.4.2 For each sample, prepare one aliquot or permanent slide mount for analysis following SOP AB-03 for periphyton prep, SOP AB-02.01 for diatom prep, or SOP AB-05 for phytoplankton prep. Code aliquots or slides A-C so that the analyst cannot determine the identity of the individual sample. Aliquots and/or slides will be referred to as samples in the remainder of this SOP.

Note: Once a year, a single sample will be split into two subsamples:

For a diatom sample, make two permanent slides from different aliquots. For a soft algae sample, prepare two counting chambers, using chambers of identical size and depth. Pairs of slides should have identical dilution.

4.4.3 Every analyst identifies each of the three samples twice for a total of six analyses following SOP AB-03 for periphyton, SOP AB-05 for phytoplankton, and SOP AB-03.01 or AB-05.1 for diatoms. Record each analysis (identifications and counts) on a separate QC data sheet labeled by sample code and analysis number.

Note: Perform analyses on each of the three samples before performing the second analysis of the three samples. Do not refer to the first analysis of the same sample when performing the second analysis. Each analysis is designed to be independent.

4.4.4 In Excel enter the data from the analyses by entering a cumulative list of taxa found in the first column and entering the unit counts from each analysis into a separate column. Code each column at the top by sample, analyst and replicate, using a single cell. Each data row should contain a unique taxon. Enter zero (0) for each sample where a taxon present in one analysis is absent in another. Insert a row at the top of the spreadsheet and enter a title detailing the date and the exercise in the top left cell (A1). Using the title created, save the spreadsheet to: \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\QCSpreadsheets\Quarterly_QC.

Note: Examples of a unique analysis code for column headings would be A-MH-1 or C-MC-2.

Note: Choose a title and file name that clearly identifies the QC exercise. An example of a title cell might be: Quarterly QC: samples EVGSLOU1234, WakSprg123 and RIVOAKTST, Analysts Hua, Chen and Dasher, May 23, 2003. Add additional information as needed.

4.4.5 Conduct Bray-Curtis similarity analyses in PRIMER-E (per SOP AB-15) with species- and genus-level taxa lists with and without square root transformation to determine the optimum percent similarity between counts. Similarity between replicate counts of a single sample by a single analyst (intra-sample variability) and similarity between counts of the same sample by each pair of analysts (inter-analyst variability) will be examined. If similarities are >70%, the QC exercise has passed.

4.4.6 If the intra-sample or inter-analyst similarities do not pass the 70% cutoff at species or genus level with square root transformation, conduct SIMPER analyses (per SOP AB-15) to determine which taxa contributed the most to the differences between counts.

4.4.7 After examining the similarity matrices, assign analyst and sample factors to the data in PRIMER-E and conduct ANOSIM analyses (per SOP AB-15) on the similarity matrices with the most passing tests.

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4.4.8 If the criteria of the ANOSIM analyses are not met as specified in SOP AB-15, taxa lists and results of the SIMPER analysis should be examined to determine the causes of the differences, and further training and discussions should be targeted in those areas.

5. QUALITY CONTROL AND DOCUMENTATION

5.1 Use a pen with black ink for all logbook and benchsheet entries.

5.2 FDEP Laboratory Information Management System (LIMS)

5.3 Phytoplankton and Periphyton Algal Identification QC Log

5.4 QC benchsheets

5.5 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error and initial the correction. If the correction requires an explanation, write a comment and reference the error.

5.6 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create a NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

5.7 See the FDEP Laboratory Quality Manual Bureau Quality Manual

6. REFERENCES

6.1 FDEP Laboratory Quality Manual, http://www.floridadep.org/labs/library/progplan.htm

6.2 SOP AB15 – Biological Community Comparisons using PRIMER_E Software

Appendix of Changes:

03/17/2008 Combined the analysts' portions of AB09, AB09_01, AB09_02, and AB09_03 to create AB-14-1.0. Updated file names and location references for bench sheets and ongoing taxa lists. Added requirement for documenting ongoing proficiency. Added requirement to include date as part of filename for QC data files. Removed the requirement to keep a spreadsheet of results from the Monthly QC (formerly called Monthly Round Robin). Added Scope and Application, Summary, and Appendix of Significant Changes.

01/25/2011 Re-organized layout to reduce redundancy.

03/25/2015 Formatted for 508 compliance. Updated links.

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