Biological Community Comparisons using PRIMER-E Software

1. SCOPE AND APPLICATION

1.1 This SOP describes analyses to compare taxa lists using PRIMER-E software. These analyses are conducted for Algal Taxonomy QC exercises described in SOP AB-14 but can be used for any investigation in which taxa lists (algae or macroinvertebrates) are compared.

2. SUMMARY OF METHOD

2.1 Bray-Curtis similarity indices are calculated to determine the percent similarity between two or more taxa lists and counts at the species- or genus-level.

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 7, but instructions are very similar for other versions.

3.2 Microsoft Excel software

3.3 Taxa lists

4. DATA ANALYSIS PROCEDURES

4.1 Import Excel QC data sheet.

4.1.1 Open the Primer-E program. Import the QC spreadsheet into Primer using File\Open. When the "Open" window appears, change "Files of type:" to "Excel files (*.xls)". Choose the file to open and either double-click on the file name or highlight it and click "Open."

4.1.2 In the "Excel Files properties" window make sure the "Data type" radio button highlighted is "Sample data." For PRIMER 6, select the worksheet to import from the drop-down menu, Data type●Sample data. Click Next>. Then, √ Title, √ Row labels, Orientation●Samples as columns, Data type●Abundance, Blank= ●Zero. Click Finish.

4.1.3 Perform Bray-Curtis similarity (section 4.2).

4.2 Calculation of Bray-Curtis similarity for species- and genus-level taxa lists.

Bray-Curtis similarity is a measure of how similar data sets are to each other. Bray-Curtis similarity has a range of 0-100 and is affected by both taxa present and their relative abundance.

4.2.1 Choose Analyze from the main menu then select Resemblance. When the "Resemblance" window opens, choose the "Samples" radio button under "Analyze between" and the "Bray-Curtis Similarity" radio button under "Measure". Click "OK". When the WARNING dialogue box appears stating no overall transform or weighting has been applied click "OK" again.

Note: The output will appear as a triangular similarity matrix, with the separate analyses as row and column headers. Each inter-analysis comparison appears once in the matrix.

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For three samples, two replicate analyses, and three analysts, there will be 18 analyses in the data file and 306 similarity coefficients in the similarity matrix.

4.2.2 A results sheet and a similarity matrix will be generated. Examine the similarity matrix. For comparisons between two samples (initial demonstration of proficiency or ongoing QC), there will be only one similarity value and this value should be >70%. For comparisons between multiple samples and analysts (quarterly QC), intra-sample (same sample analyzed twice by one analyst) and inter-analyst (same sample analyzed by different analysts) similarities should be >70%. If similarities are >70%, the QC has passed. Record the similarity value in the original Excel file.

Note: A target similarity value of 70% was chosen because proficient analysts routinely exceed a Bray-Curtis value of 70% in inter-aliquot comparisons from the same sample.

4.2.3 If Bray-Curtis similarities are below 70%, make the original taxa list the active worksheet. Click Pretreatment\Transform(overall) and select from the drop-down menu to perform the square-root transformation. Then follow steps in 4.2.1 to get to Bray-Curtis similarity. If similarities are >70%, the QC has passed with square root transformation. Record the similarity value in the original Excel file and note that square root transformation was required.

4.2.4 If the 70% cutoff is not met in step 4.2.3, copy the Excel spreadsheet with the species list, keeping the same title but add genus and delete species from the title. Open the genus file and carefully combine all the taxa of the same genus under one name, their genus name with "sp." Double check to make sure you added the rows correctly by checking the total in the original species file against the total in the genus file. Repeat steps 4.2.1 with the genus file. If the similarity value is below 70% then repeat step 4.2.3 with the genus file.

Note: The goal of combining taxa to the generic level is to determine if analysts differ in level of identification (i.e. species vs. genus or genus vs. family).

4.2.9 Using the new spreadsheet generated in Primer at the generic level, repeat calculation of Bray-Curtis similarity following steps 4.2.1 to 4.2.3. If the similarities are >70%, without or with transformation at the generic level, the QC has passed. When recording the similarities in the original Excel file, note that the QC passed at the generic level but not species level.

4.2.10 If the similarities do not pass the 70% cutoff at any level with square root transformation, compare the differences between taxa lists. Those taxa contributing the highest percentage of dissimilarity between counts should be examined to determine the source of the discrepancy (count, level of identification, etc.).

Appendix of Significant Changes:

03/17/2008 Created SOP AB-15-1.0 from portions of SOPs AB09, AB09_01, and AB09_03 that contained instructions for PRIMER analyses. Added updated commands for PRIMER v6.0.

04/15/2011 Added step 4.1 to import a file; re-numbered remaining sections. Added directions for PRIMER 6.

03/25/2015 Formatted for 508 compliance.

09/20/2018 No changes made.

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01/16/2020 Updated SOP for Primer 7; aggregate files no longer created to perform tests and not yet able to determine how to perform them in Primer 7; removed SIMPER and ANOSIM tests—not currently being performed and not yet able to determine how to perform them in Primer 7.

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