STREAM CONDITION INDEX REFRESHER AUDIT LAB AUDIT REPORT

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Scope of Audit

Samplers or field organizations that submit Stream Condition Index (SCI) data to the Florida Department of Environmental Protection (DEP) must pass an initial field audit and then pass a refresher audit every five years, per DEP SOP SCI 1300. The refresher audit requires the sampler or field organization to collect an SCI sample at a designated DEP SCI Benchmark Site and submit the sample to a laboratory for processing, which includes lab sample sorting, lab taxonomic identification, and calculation and reporting of the SCI index resulting from the sample analysis. The sampler or field organization is designated with a "pass" status in the DEP Bioassessment Registry if the reported SCI score is no more than 19 points lower than the median SCI score for the Benchmark Site. An SCI result that is within the acceptance criteria established for the respective benchmark site provides assurance to DEP that both the sampler or field organization and the taxonomic lab are properly following the DEP SOP. To provide further assurance that the macroinvertebrate taxonomy lab is following the sample processing and index calculation protocols in the SOP, DEP conducts an audit of the documentation generated from the refresher audit sample analysis to better determine how closely the lab complies with the procedures in DEP SOP SCI 2000. Note that the procedures and quality control for sorting and taxonomic identification assessed during this audit are the same as those required for the macroinvertebrate Wetland Condition Index (DEP SOP LT 7600).

Audit Outcome

DEP found that the Water and Air Research macroinvertebrate taxonomy lab procedures and results are in accordance with DEP SOP SCI 1000 for sample sorting, taxonomic identification, and index calculation.

Field Sampling Organization /Sampling Personnel:	Water and Air Research / Laura Line and Efrain Tavarez
SCI Benchmark Site:	Little Orange Creek
Sample Collection Date:	5/22/12
Macroinvertebrate Lab(s):	Water and Air Research (WAR)
Analysis Date(s):	5/30/12 – 6/5/12
Auditors:	Nia Wellendorf and Joy Jackson, DEP
Records Provided Date:	3/12/13
Audit Report Date:	8/15/13 (initial), 10/25/13 (final)
Copies of Final Audit Report to:	David Evans (WAR), Laura Line (WAR), Efrain Tavarez (WAR), Russ Frydenborg (DEP), Kevin O'Donnell (DEP), Michael Blizzard (DEP)

Records Evaluated

The following records (with file names) were submitted to DEP by WAR and evaluated for this audit:

- Sample sorting bench sheets (SCI Sort and QA Sheets W&AR Little Orange Creek.pdf),
- Taxonomic identification bench sheets (SCI Test Bench Sheets W&AR Little Orange Creek.pdf),
- Description of corrective actions (Corrective Action.doc),
- Taxonomic keys most frequently used (References most used for FL Freshwater Taxonomy.doc),
- List of verified taxa in reference collection (Water & Air Freshwater Verified Taxa.xls),
- QC records for sorting and taxonomic identification for 2012 (FDEP – W&AR QA records for Sorters.xls, FDEP – W&AR Master List – Benthic Taxonomist QA Record.xls),
- List of SCI samples analyzed in 2012 (SCI List for 2012 W&AR.xlsx),
- Collapsed taxa lists and taxonomic attributes for metrics (Raw Data and Taxa used in SCI Metrics Little Orange Creek W&AR.xls),
- Metric values and SCI score value (Little Orange Creek SCI Indices [Unpooled].pdf).

Audit Findings

***Lab Quality System*:** The Water and Air Research (WAR) lab conducts all quality control (QC) measures as required by the SCI SOP.

***Sample Sorting and Identification Process*:** Overall, the sorting and identification process follows the DEP SOP. Samples were properly homogenized, two replicates were picked, and the proportion of sample picked was properly recorded. Although not classified as a deficiency, one recommendation for improvement involves the techniques used for achieving the target number of organisms during the sorting process.

For replicate 2 of the refresher audit sample, the technician picked 161 organisms from the sample, which is 1 greater than the target range of 140-160. The SOP states that 150 individuals should be randomly picked out from the sorted organisms until the target number of organisms is reached. A random number table was used to select organisms to be removed from the replicate after taxonomic identification. For this particular sample, 11 individuals were removed from the replicate, which is consistent with the SOP.

Picking one organism greater than the target range for a SCI replicate is not problematic at all, and the follow-up action by WAR for the refresher audit sample was appropriate. However, the sorting QC records show several SCI samples for which many more than 160 organisms were picked (177, 196, 199). These high numbers suggest that the sorters were not consistently effective at reaching the target number. For the refresher audit sample, detailed sorting records from Replicate 2 indicate that the sample material was sufficiently homogenized. DEP recommends that technicians pick a single square at a time or a half square if needed to achieve the target; technicians should document the actual fraction picked. This overpicking is not truly a deficiency because the SOP allows for organisms to be removed if more than 160 organisms are picked; however, it would be more efficient to achieve the target without having to use the additional removal step.

For the refresher audit sample, two different technicians sorted the two sample replicates, and they differed in the details they documented about the sorting process. One technician (ET) documented the sample fraction and number of organisms picked from each aliquot, while one technician (MRP) only documented the total fraction of the sample sorted and the total number of organisms picked from the replicate. Both technicians captured the fraction of the replicate picked and the number of organisms picked; however, the former (ET) was more consistent with the SCI SOP requirement to document the "total number of organisms sorted from each selected grid tray aliquot" (DEP SOP SCI 2110). The latter technician's (MRP) omission of the aliquot level information did not prevent accurate calculation of the SCI score, but was inconsistent with the SOP.

***Sample Sorting QC**:* WAR conducts a QC check for sorting on a full replicate sample for 10% of all SCI samples, whereas the SOP requires a QC check on 10% of sorted aliquots for each SCI

replicate. Both methods of sorting QC are acceptable, and DEP does not recommend any change to the WAR sorting QC procedure.

***Taxonomic Identification QC**:* The WAR laboratory taxonomic identification procedures and quality control evaluations of individual taxonomist performance meet DEP SOP requirements. WAR has several taxonomists on staff, so they confer amongst themselves as needed for identifications, and conduct taxonomic QC on at least 10% of samples. The SCI sample list provided to DEP did not include analyst names for each sample; therefore, the DEP auditor was not able to determine the specific QC percentage per analyst. However, the number of QC samples in 2012 was 13% of the total samples (combining SCI and WCI samples). Based on a DEP taxonomist's review of the taxa list, WAR taxonomists achieved the appropriate taxonomic level as directed by the SCI SOP.

***SCI Index Calculation**:* WAR correctly classified taxa according to their attributes, and correctly calculated the ten SCI metrics and final SCI scores for each replicate according to the DEP SOP.

More detailed audit findings are contained in the audit checklist on pages 4 and 5.

Corrective Actions

Although not classified as a deficiency, DEP recommends additional training for SCI sample sorting technicians to more consistently achieve the 140-160 organism target. Sorting technicians should routinely record the number of organisms sorted from each selected grid tray aliquot.

Lab Records Audit Checklist for SCI sampling processing and data analysis			
Lab	Water & Air Research		
Sample	SCI Refresher Audit sample, sampled 5/22/12 by Water & Air Research, Little Orange Creek		
PERFORMANCE MEASURE		**PERFORMANCE ASSESSMENT**	
		Assessment	**Remarks**
1. Lab quality system (SCI 2200, LQ 7400 1. General Requirements) - *Could apply to SCI, BioRecon, LCI, WCI, Hester-Dendy, Dredge, or Core sample analysis*		***Refer to list of taxonomic keys and list of reference collection specimens to address the following requirements.***	
1.1	Lab maintains copies of appropriate taxonomic identification keys.	YES	Merritt and Cummins was missing from the list, but Laura Line confirmed that they have that reference. The damselfly key on the list was not the most current version; WAR taxonomists should access the newer version on the DEP website. http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/dragonflykey.pdf
1.2	Lab maintains taxonomic specimen collection *(SCI 2200 section 1)*.	YES	
1.3	Taxa found in samples are represented in the reference collection *(SCI 2200 section 1)*.	YES	Checked taxa from 5 orders and verified that they were on reference collection list.
1.4	Lab retains extramural experts to verify identifictions *(SCI 2200 section 2)*.	YES	
1.5	Specimens in collection are verified by extramural expert *(SCI 2200 section 1)*.	YES	Note that some columns seemed out of alignment or some specimens did not have a verification taxonomist listed (see Coleoptera worksheet).
1.6	Lab contains basic equipment necessary for sorting and taxonomic analysis (compound and dissecting microscopes, divided trays, #30 sieve) [applicable to lab	N/A	Auditor did not visit the lab, so this element was not evaluated.

	visit audit only]		
2. Sample sorting and identification process (SCI 2100) - *Applies to processing for SCI samples, could be adjusted for other samples that follow LT 7010-7040*		***Refer to raw bench sheets to address the following requirements.***	
2.1	Sample homogenized and subdivided at prescribed levels; records indicate fractions of samples sorted and reflects that 2 or 3 trays were used (may only have 2 if insufficient material to warrant 3 trays) *(SCI 2100 Sections 3.1.2.5 - 3.1.2.9, 3.1.2.14)*	YES	Fractions of sample picked were 7/288 for Rep 1 and 1/32 for Rep 2 as listed on taxonomic ID bench sheets, and those fractions match what is on the sorting bench sheets.
2.2	Two aliquots of 150 (±10) organisms picked from sample material *(SCI 2100 Sections 3.1.2.8 - 3.1.2.16)*.	YES	There is some deviation from SOP in achieving the 150 organism target. When the number exceeded 160 (161), WAR staff randomly picked out 11 individuals using a random number generator and then removed those individuals. Per SOP LT 7200 3.1.2.12, the number of individuals should be reduced right after sorting and before taxonomic identification. In the future, the target number of organisms (140-160) should be acheived within the sorting process and before ID. Sorting records show several samples for which many more than 160 organisms were sorted (177, 199, 196).
2.3	The X qualifier is used if insufficient organisms are in the sample to obtain 2 replicates of 150. (only applicable if <300 organisms were in the sample) *(SCI 2100 Sections 3.1.2.17)*	N/A	Sufficient material for two reps of 150, so this element was not evaluated.
2.4	Analysts prepare slides and use a compound microscope for identification of appropriate groups when needed (typically worms, midges, mites) *(SCI 2100 Section 3.2)*	YES	
2.5	Individual taxon names and counts are recorded on benchsheet *(SCI 2100 Section 3.3)*	YES	
2.6	Slides clearly identified and linked to the sample vials and sample data sheets by unique sample ID and specimen ID *(SCI 2100 Section 3.2.2.2)*	YES	Bench sheet have slide numbers designated for specimens. Slides not examined for audit, but Laura Line of WAR said that each slide is labeled with sample IDs, dates, and aliquot information sufficient to provide linkage between slides and other sample records.
2.7	Taxonomic benchsheet indicates when specimens are removed from sample vial for any reason (e.g., placed in reference collection or send to extramural expert) *(SCI 2100 Section 3.3.2.6)*.	N/A	None noted, so this element was not evaluated.
2.8	Taxa collapsing routine conducted per SOP SCI 2100 section 4. or LT 7040. Original and collapsed taxonomic lists maintained.	YES	
2.9	Documentation available for items listed in SCI 2110	NO	Some items on sorting bench sheet were incomplete or inaccurate; for Rep 1, some QC information was entered into the wrong boxes, and the number of individuals sorted from each aliquot was not recorded.
3. Sample sorting QC (SCI 2210, LQ 7410) - *Applies to processing for SCI, LCI, WCI, Hester-Dendy, dredge or core samples*		***Refer to sorting efficiency tracking records, corrective action protocol, list of analyzed samples, and raw bench sheets to address the following requirements.***	
3.1	Sorting efficiency check conducted for a minimum of 10% of aliquots per sample *(SCI 2210 Section 1)*.	YES	WAR conducts sorting QC checks on full replicates (i.e., all sorted aliquots for a selected sample replicate are checked) for 10% of samples, whereas the DEP SOP only requires sorting checks for (a minimum of) 10% of sorted

			aliquots for all replicates. The sorting QC procedure used by WAR is acceptable, and is more rigorous than the DEP SOP sorting QC requirement.
3.2	Sorting efficiency calculation conducted per SOP *(SCI 2210 Section 1.4)*	YES	WAR calculation uses a different formula, is based on checking all of the sorted aliquots for the selected sample replicate, and is more rigorous than the SOP requirement.
3.3	Sorting efficiency tracked for each analyst *(SCI 2210 Section 2)*.	YES	Sorting check conducted for SCI Refresher sample, but that sample was not on the running list of sorting QC for either sorter (MRP or ET) because it was not a standard project. All samples should be included in analysts' QC records, regardless of project type.
3.4	Cumulative sorting efficiency 95% or greater for each analyst *(SCI 2210 Section 2)*.	YES	
3.5	Precautionary measures taken when single-analyst cumulative sorting efficiency falls below 95% *(SCI 2210 Section 2)*.	YES	
3.6	Corrective action taken when single-analyst cumulative sorting efficiency falls below 90% *(SCI 2210 Section 2)*.	YES	Corrective action when below 95%.
3.7	Documentation available for items listed in SCI 2211	YES	
4. Taxonomic identification QC (SCI 2220, LQ 7420) *- Applies to processing for SCI, LCI, WCI, Hester-Dendy, dredge or core samples*		***Refer to taxonomic QC tracking records, corrective action protocol, list of analyzed samples, and raw bench sheets to address the following requirements.***	
4.1	For freshwater sampling, identify taxa to the lowest practical taxonomic level as listed in Table LT 7900-1 or SCI 2230. *(SCI 2100 Section 3.3.2.4)*	YES	Joy Jackson (DEP taxonomist) reviewed the taxa list and found the taxonomic levels to be acceptable and consistent with the DEP SOP requirement and guidance.
4.2	Quality control performed on 5% of samples (or SCI replicates), per SCI 2220 section 3 or LQ 7420 section 3.	YES	Documentation provided by WAR indicate that each taxonomist conducted at least one QC during the year. A total of 7 replicates were QC checked of 52 total replicates analyzed in 2012 (SCI and WCI combined), which yields QC check of 13% of samples. Laura Line (WAR) indicated that their intent is to conduct taxonomic QC checks for 5% of samples.
4.3	Taxonomic discrepancies associated with the QC check resolved through use of extramural expert when needed. (may only be able to evaluate this element if corrections were made on bench sheet) *(SCI 2220 section 3.4)*	YES	WAR also has several taxonomists in house, and the taxonomists commonly consult amongst themselves.
4.4	The single-analyst cumulative correct identification rate is 95% of the total number of individual organisms and 95% of taxa identified in all samples or subsamples enumerated by the analyst (5% cumulative error rate over 12 months) *(SCI 2220 section 2)*	YES	WAR QC guidance sheet indicates a 90% correct identification threshold, but all analysts have met the 95% DEP SOP requirement. WAR should update internal requirements to be consistent with SCI 2220.
4.5	Documentation available for items listed in SCI 2221.	NO	Errors are only tracked by WAR based on the number of individuals, but should also be tracked for taxa.
5. SCI Index calculation (SOP SCI 2100 section 5.) - *Applies to SCI only. Additional checklist would be needed for other indices.*		***Refer to SCI metric and index calculation records to address the following requirements.***	
5.1	Taxa correctly labeled as long-lived, sensitive, and very tolerant per SOP SCI 2100 section 5.1-5.3	YES	

5.2	Taxa correctly assigned to the following taxonomic groups, as required for metric calculations in SOP SCI 2100 section 5.4: Ephemeroptera, Trichoptera, Tanytarsini tribe	YES	
5.3	Taxa correctly labeled as having a clinger or filter feeder habit, per Merritt and Cummins, An Introduction to the Aquatic Insects of North America *(SCI 2100 section 5.5)*	YES	There was one error in the attributes sent to DEP. All taxa were accurately categorized with the following exception: Thienemannimyia group sp. Epler was considered a Filterer, but is a predator and not filterer in Merritt and Cummins 1996 (and not on DEP list of filterers). However, the %filterer calculation did not include that taxon as a filterer, so there was just an error in the taxa list and attributes sent to DEP and not in the metric calculation (Laura Line of WAR confirmed that this was just an error in the documents sent to DEP and not in their database). Note that all Elmidae will be considered clingers per revised QA Rule, not yet effective (2008 Merritt et al.).
5.4	Sample correctly assigned to SCI region *(SCI 2100 section 5.6)*	YES	
5.5	Metrics and index calculated for each replicate following instructions in SOP SCI 2100 Table SCI 2100-1 and steps 1-6 following the table.	YES	Metric calculations verified as correct per effective 2008 SOP. Taxa with more than one habit are correctly included as 1/2 individuals in the %filterer calculation; metrics correctly trimmed.

	Performance Assessment Remarks	WAR Laboratory Explanation and/or Corrective Action	Approved by AEQA auditors
2.9	Some items on sorting bench sheet were incomplete or inaccurate; for Rep 1, some QC information was entered into the wrong boxes, and the number of individuals sorted from each aliquot was not recorded.	The technicians are being trained to record the number of organisms from each aliquot and to check that numbers are entered into the correct boxes	10/25/13
4.5	Taxonomic QC errors are only tracked by WAR based on the number of individuals, but should also be tracked for taxa.	Taxonomic QC errors are tracked on the individual taxa QC sheets. However, we have added a column to the tracking excel spreadsheet to include a place for taxa missed.	10/25/13