Final Audit Report

Robert P. Rutter

Stream and Wetland Condition Index Laboratory Audit

Aquatic Ecology and Quality Assurance Section
Water Quality Standards Program
Division of Environmental Assessment and Restoration
Florida Department of Environmental Protection
October 2017

STREAM AND WETLAND CONDITION INDEX

LAB AUDIT REPORT

Scope of Audit
Robert P. Rutter has provided Stream Condition Index (SCI) data to the Florida Department of Environmental Protection (DEP or department) as a sub-contractor for Ardaman & Associates, Inc. in support of a Numeric Nutrient Criteria (NNC) compliance demonstration for US Agri-Chemicals Corporation, and SCI and Wetland Condition Index (WCI) data as a sub-contractor for Tetra Tech, Inc. for a Site Specific Alternative Criteria (SSAC) for pH for the Panama City Beach Wastewater Treatment Plant. To provide assurance that Mr. Rutter follows the sample processing and index calculation protocols in DEP SOPs SCI 2000 and LT 7000, the DEP Aquatic Ecology and Quality Assurance Section (AEQAS) conducted a records audit of the required documentation associated with a subset of sample results submitted by Mr. Rutter as part of these projects.

Three samples, processed between May 2015 and January 2017, were reviewed for adherence to sample preparation and identification protocols required in LT 7000 and SCI 2000. Records from the following samples were reviewed as part of this audit:

- Panama City Beach - WCI REF- 04, May 6, 2015
- Ft. Meade – SCI, Mill Branch, October 20, 2016
- Ft. Meade – SCI, Mill Branch, January 20, 2017

Some of the audit findings are unique to the individual samples, while other findings and comments pertain to the overall laboratory protocols for the handling and processing of SCI and WCI samples.

Summary of Findings/Acceptance of Corrective Actions
A preliminary audit report was sent to Mr. Rutter on July 25, 2017, and Mr. Rutter provided responses to the preliminary report on August 11 and 25, 2017. The findings, Mr. Rutter's responses, and the department's comments are provided in Table 1.

The department found certain lab sorting procedures performed by Mr. Rutter differed from DEP SOP SCI 2000. However, a further review of the taxa lists, calculations, and final scores demonstrate that the procedures resulted in well homogenized samples. The taxonomic identification and index calculations comply with DEP SOPs. Mr. Rutter has provided acceptable responses to the listed findings.

Lab Quality System: Mr. Rutter maintains copies of pertinent taxonomic identification keys and primary literature and an extensive reference collection (~1,075 specimens), over half of which have been verified by external experts. The reference collection contained specimens of all taxa found in the Mill Branch (10/20/16) sample. Representative organisms from the other two samples were checked by the auditor, and all organisms checked were represented in Mr. Rutter's reference collection.

Sample Processing, Sorting and Quality Control (QC): Examination of the laboratory documentation requested for the audit indicates that Mr. Rutter's sample processing protocols follow DEP SOPs with some modifications. The SCI sample processing logs indicate that the detrital matrix was spread across a tray with 96 grids and that individual aliquots were taken from this tray. DEP SOP SCI 2100 Sections 3.1.2.6. and 3.1.2.7. require randomly removing 1/3 of the squares from the first tray, mixing it thoroughly and placing it into a second tray, and then randomly selecting grids from the second tray, to be placed

into a third tray. It is from this third tray that individual aliquots are sorted to obtain the target number of individuals. While Mr. Rutter's methodology did not conform to the steps as specified in the SOP, it did ensure that each organism had an equal chance of being selected and did not likely affect the analytical results or the calculated SCI scores for the audited samples.

The average cumulative sorting efficiency was not initially provided, but was submitted in response to the preliminary audit report. No sample fell below the 95% sorting efficiency; thus, the sorting target was met.

A brief written description of Mr. Rutter's laboratory taxonomic QC results was initially submitted, but not the result of the taxonomic QC for Mill Branch (Rep 2) from October 20, 2016, or the cumulative identification rate. DEP requested this information, and the results show that the taxonomic correct identification rate was 86%. The target rate is 95%. Mr. Rutter indicated that the following corrective actions were taken due to the results of this QC sample: appropriate taxonomic literature and reference specimens were reviewed, the customer was notified of discrepancies, companion samples were re-identified and corrected as necessary, and the SCI scores were re-calculated. The taxonomic QC checks were not being performed at the specified rate, which is 5% or at least one sample per year.

SCI and WCI Index Calculation: There were minor rounding differences between the calculations performed by the analyst compared to the Statewide Biological Database (SBIO) calculations. However, these differences did not result in a difference in the replicate scores (56 and 73) or the final score (64) compared to the SBIO calculations. As part of the Panama City Beach Wastewater Treatment SSAC study, a previous review was conducted of the WCI calculations by DEP. The main discrepancy noted during that review was the inclusion of the amphipod, *Crangonyx* sp., as a Florida Index taxon by Mr. Rutter. *Crangonyx* sp. is not a Florida Index taxon and should not have been counted as such. While this discrepancy did not result in a different final score, calculations were corrected in that and other WCI samples.

Data Usability Recommendation

Based on the results of this audit, AEQAS recommends that the SCI and WCI data produced by Mr. Rutter be used for SSAC and NNC compliance purposes. Corrective actions will not be implemented pursuant to this audit because the analyst is retiring and will no longer be processing macroinvertebrate samples.

Table 1. Audit findings of deficiency for the Laboratory Records Audit of selected SCI and WCI sample data produced by Robert P. Rutter, conducted by the Florida Department of Environmental Protection Aquatic Ecology and Quality Assurance Section.

SOP reference	Audit Finding	Robert P. Rutter Response	DEP Comments
SCI 2100 Section 3.1.2.5 – 3.1.2.9	The detrital matrix is initially spread into 96 grids from which individual grids are selected rather than as instructed in the SOP. The SOP discusses placing the material into 24 squares from which 1/3 is randomly selected and placed into a second tray. Individual squares are randomly removed and placed into a third tray to achieve the target number of individuals. This practice does not achieve the same degree of homogenization as specified in the SOP. One-third of the original 24 grids of the material should be removed and further homogenized, ensuring further mixing that may not have occurred during the rinsing step.	Washed and reduced sample is spread to a reasonable thickness across 24 primary grids (or less if there is insufficient material). Each primary grid is divided into four secondary grids (within the same tray), totaling 96 grids. Secondary grids can be further sub-divided to achieve target count. While the analyst agrees that current process may not achieve the same level of homogenization as specified in the SOP, it does ensure that every critter has an equal chance of being selected.	A review of the taxa lists suggests that the washing and reducing conducted by the analyst resulted in well homogenized samples. No organisms appeared to be overly dominant as might occur with insufficient mixing. DEP does require adherence to SOPs; however, because the analyst is retiring and will not be processing any additional samples, no corrective action will be taken.
SCI 2100 Section 3.1.2.11-3.1.2.12	Auditor was unable to determine how the final target number of individuals was obtained when it was initially exceeded.	The Sample Processing Log records the initial sorting total count, which from my experience is almost always higher than the total Bench Sheet count after the sample has been identified. This can be due to several things, including worm fragments; items that upon closer examination are not relevant (e.g. rootlet, empty shell, terrestrial organisms). Thus, it was not necessary to reduce sample numbers.	This response is acceptable. No corrective action is required.

SOP reference	Audit Finding	Robert P. Rutter Response	DEP Comments
SCI 2100 Section 3.2.2.2	Auditor was unable to follow the handling of slide-mounted organisms through the laboratory's process based on the documentation provided. Please clarify the differences in information contained on the lab processing form and the bench sheet. It was unclear what was meant on the sample processing logs under the slide mounted section by "Worm 1-9/slide" and "Midge 1-6/slide" and how this related to the number of slide-mounted organisms in the sample	As slides were mounted they were placed on a tray and each set of slides was grouped and numbered and indicated as such on the (Macroinvertebrate Mounted) Bench Sheet.	In subsequent communication with the analyst, the auditor later determined that the nomenclature (Worm 1-9/slide) referred to the range of numbers that may be mounted on a given slide and otherwise does not reflect the number of those specimens in the sample. This response is acceptable. No corrective action is required.
SCI 2210, Section 1.5	Less than 10% (8.5%) of the aliquots for sample REF-04 were selected for Sorting QC. Other samples reviewed had the required minimum. It appears this was a transcription error on the first line of the Sorting Efficient QC Log where it states that 47 aliquots were sorted for REF-04 and 4 dishes were selected for QC. This should have been 38 as indicated on the WCI Sample Processing Log.	Regarding sample REF-04, my records indicate that 38 petri dishes were sorted and four were selected for QC = 10.5%. Sorting Efficiency QC Log was corrected.	This corrective action is acceptable and considered complete.
SCI 2211	The cumulative sorting efficiency per analyst was not provided. Please provide this information.	Records were provided.	Analyst provided the requested records in "Audit Finding #5: Cumulative Sorting Efficiency," and sorting efficiency was ≥95%. This corrective action is acceptable and considered complete.
SCI 2220, Section 3.6 and SCI 2221 Section 1.1 – 1.2	The cumulative identification rate for the number of organisms and the number of taxa were not provided for the analyst. Please provide this information.	The significant errors concerned *Labiobaetis propinquus* and *C. hilaris*, prompting me to re-visit the appropriate keys and my reference specimens. I notified customer of the discrepancies, and re-identified and corrected as necessary the companion samples, and re-calculated the SCI's.	Analyst provided records in "Audit Finding #6 Cumulative Identification Rate," and auditor calculated the identification rate as 86% (error rate = 14%) based on the individuals in a single QC sample processed on 1/19/17.

SOP reference	Audit Finding	Robert P. Rutter Response	DEP Comments
SOP 2220 Section 3.1	QC checks were not performed at the prescribed rate of 5% of the samples or at least one per year. Three years of data were reviewed, and only one QC sample was performed.	This was the only QC sample available and no future QC samples will be performed as analyst is retiring and will not be processing samples in the future.	DEP requires more frequent QC samples; however, because the analyst is retiring and will not be processing any additional samples, no corrective action will be taken.