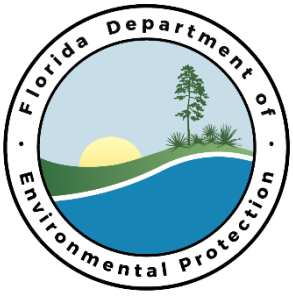
Final Stream Condition Index Laboratory Audit Report

McGlynn Laboratories, Inc.



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

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2600 Blair Stone Road, MS 6511
Tallahassee, Florida 32399-2400
www.dep.state.fl.us

STREAM CONDITION INDEX LAB AUDIT REPORT

Scope of Audit

McGlynn Laboratories, Inc. (McGlynn Labs) has provided Stream Condition Index (SCI) data to the Florida Department of Environmental Protection (DEP or department) in support of the City of Marianna's Wastewater Treatment Plant (WWTP) biennial monitoring required by permit (FLA101141-008-DW1P/NRL). It came to the attention of DEP that McGlynn Labs had not maintained its SCI proficiency status required by DEP SOP SCI 1300, Section 1.3. The SOP requires that entities submitting SCI data to DEP undergo a refresher audit every five years. To provide assurance that McGlynn Labs was following the sample processing and index calculation protocols in DEP SOP SCI 2000, DEP conducted a records audit of the required documentation associated with samples from two monitoring sites, SWD-02 in Jack Creek and SWD-04 in Pumpkin (Mill) Creek, collected on July 5 and 8, 2016. As part of this audit, records for the two SCI samples were reviewed for adherence to sample preparation and identification protocols required in DEP SOP SCI 2000.

A preliminary audit report was sent to McGlynn Labs on June 21, 2018, and McGlynn Labs provided responses to the preliminary report on August 2, 2018 (Table 1). Those findings are summarized in the *Records Audit Findings* section of this report. The responses did not provide DEP with assurance that the issues identified were fully understood or corrected.

Given the unresolved issues, DEP issued a revised preliminary report on September 13, 2018, that outlined additional steps McGlynn Labs must take to demonstrate full compliance and understanding of the pertinent SOP sections, as outlined in Corrective Actions 1-4 below and in Table 1. This final report documents which of the additional steps were undertaken and which have not been fully implemented.

Corrective Actions

The items listed below were identified by DEP as essential for demonstrating that that the corrective actions in Table 1 had been properly implemented:

1. Seek additional field and laboratory training.
2. Demonstrate SCI sampling proficiency as described in SCI 1300.
3. Update the McGlynn Laboratory's internal quality manual to reflect adherence to the January 2017 SCI SOP and implement corrective actions listed in Table 1.
4. Undergo a laboratory audit by DEP confirming that the items in Table 1 have been correctly and fully implemented.

As of April 2019, McGlynn Labs has completed one (1) of the four (4) Corrective Actions (he sought training).

Corrective Action #1. On September 6, 2018, Dr. McGlynn visited the DEP Central Laboratory to observe the SCI sample processing procedures. Dr. McGlynn observed an experienced analyst use all the relevant safety and laboratory equipment to prepare an SCI sample for taxonomic identification. Dr. McGlynn observed the analyst rinse, reduce, and homogenize an SCI sample, which was then placed into appropriately-sized gridded trays from which randomly-selected aliquots were removed for sorting. Sample sorting quality control measures were demonstrated and reiterated throughout the process. In addition, Dr. McGlynn and his assistant attended the DEP Bioassessment Training event on September 25, 2018.

Corrective Action #2. Dr McGlynn was evaluated by DEP staff on his ability to properly perform SCI sampling as described in FD 9000-34. It was determined that additional effort was required by Dr. McGlynn before he was ready to receive a second in-person field audit. On September 26, 2018, an email was sent to Dr. McGlynn detailing the items needing additional attention before Dr. McGlynn was

ready for a follow-up field audit. On October 23, 2018, DEP staff emailed Dr. McGlynn asking whether he had practiced the items outlined in the September 26, 2018 email and asking about his availability to undergo a field audit to satisfy Corrective Action #2. Dr. McGlynn responded that he had not accomplished much towards the audit and he did not indicate an interest in scheduling a field audit.

Corrective Action #3. Dr. McGlynn has not provided a revised quality manual that incorporates the January 2017 SCI SOP and corrective actions pursuant to the preliminary reports.

Corrective Action #4. 1. McGlynn Lab's agreed to a laboratory audit by DEP, but the audit did not confirm that the items in Table 1 have been correctly and fully implemented. See *Lab Audits Findings* below.

Records Audit Findings

McGlynn Labs' protocols deviated in numerous aspects from the protocols specified in DEP SOP SCI 2000. Based on the findings outlined below, DEP has determined that McGlynn Labs does not currently provide accurate or reliable macroinvertebrate data. The findings include numerous deficiencies as noted throughout this report and summarized in Table 1.

***Sample Processing, Sorting and QC*:**
In response to DEP's request for sample processing and sorting records, Dr. McGlynn provided a summary of his SCI sampling protocols (SCI Sampling Summary – Dr. McGlynn -053018). According to Dr. McGlynn, he did not use gridded trays for subsampling because there were so few organisms. Dr. McGlynn mixed the contents in a sieve and then divided the contents into two subsamples. The documentation did not specify how the material was divided into the two subsamples but states that Dr. McGlynn then sorted the entire sample(s) to get the target number of individuals. It is unclear how sorting all the material results in the exact number of individuals required for the SCI calculations (150 ± 10). This was found to be the case for all the samples processed during the 2016 sampling event.

Also, according to the "SCI Sampling Summary" document, Dr. McGlynn covers the sorting pan and stores it for at least two hours. He then reexamines the pan three additional times to verify that it is completely picked. DEP interpreted this to mean that the material is not sorted aliquot by aliquot with a dissecting microscope, but rather is examined all at once in the white enamel sorting pan. Dr. McGlynn did not have any sorting efficiency records nor does he have a second analyst performing QC checks. Without these records, DEP was unable to assess Dr. McGlynn's sorting efficiency.

The Tanytarsini group is a very common group of dipterans found in SCI samples, yet no representatives of the Tanytarsini group were identified in any of the samples reviewed as part of this audit. While it is possible that none were present at the time of sampling, the lack of Tanytarsini suggests that smaller organisms may have been systematically overlooked in the sorting process. A second analyst reviewing Dr. McGlynn's sorting protocols would likely uncover any systematic issues such as missed (unpicked) organisms.

Dr. McGlynn noted that, in instances when there are too few individuals to achieve the target number, he resamples under different field conditions, if possible. However, SOP 2100 Section 3.1.2.17 clearly states that if the entire sample is processed and there are insufficient organisms to achieve the minimum (140 organisms), then an "X" qualifier must be used for reporting purposes.

***Taxonomic Identification QC*:** McGlynn Labs outsources the taxonomic identifications to John Epler, Ph.D., whose procedures have been reviewed by DEP as part of a previous audit and thus were not included in this review (OGC Case No. 15-0617). There were only minor issues noted in that audit, and the auditee agreed to the Corrective Action Implementation Plan. As such, DEP considered the responses acceptable and complete. However, an additional taxonomy-related issue was noted during the audit. Namely, the taxon *Dolomedes* sp. was present in the four replicates reviewed (SWD2-1 n=1, SWD2-2 n=2, SWD4-1 n=1, and SWD4-2 n=1), and *Dolomedes* sp. is considered semi-aquatic and is

not included in Table SCI 2230-1. Removing this organism from the samples reduces the total taxa metric by one taxon. Given how few individual *Dolomede*s sp. were in the samples, incorrectly including it had a minimal impact on the final score(s); however, in the future, *Dolomedes* sp. should not be included in taxa counts.

***SCI Index Calculation*:** There were multiple SCI calculation errors that resulted in an overestimation of the SCI scores by 10 points for SWD2 (includes SWD2-1 and SWD2-2) and 9 points for SWD4 (includes SWD4-1 and SWD4-2). The most obvious discrepancy was the use of the SCI_2007 calculations instead of the current SCI_2012 version. All the formulas were revised in the SCI_2012 version except the long-lived taxa metric. Numerous errors were also made in assigning and tallying taxa to the various metrics. For example, there were three *Molanna blenda* individuals that were counted as three separate taxa for the long-lived metric rather than as a single taxon. In addition, individuals identified as *Rhagovelia* sp., should have been collapsed into *Rhagovelia obesa* and *Rhagovelia torreyana* proportionately and not counted as a separate taxon.

Step 5.9 of the Data Reduction Section in SCI 2100 states that, after tallying the individual metrics, any metric score greater than 10 must be changed to 10. In the McGlynn Lab version of the calculations, there were three metrics that exceeded 10 (Percent Filterer, Long-lived, and Percent Dominance) and none were reduced. After the metric scores are summed, they should be divided by 0.9, and this step was not performed. Finally, the two replicates should be averaged and then rounded to the nearest integer to obtain a single, final SCI score; this step was not performed.

The following section is not typically included in an SCI laboratory records audit; however, it is important to provide feedback on items presented in the "SCI Sampling Summary" document that have the potential to influence the integrity of the SCI results.

SCI Sampling: In the materials submitted, Dr. McGlynn discussed how the streams sampled as part of the permit are all first order streams and prone to sand smothering and desiccation. According to Dr. McGlynn, the permit-specified sampling period of June, July, and August means that the streams are either dry or subject to unusually heavy rain events associated with hurricanes that can lead to habitat scouring and/or sand smothering, both of which often result in too few individuals for a valid test. According to DEP Northwest District Staff (B. Evans, personal communication), the permit no longer limits the sampling to the summer period. Going forward, the SCI should be sampled per the SCI SOP and SCI Primer documents, which clearly outline the conditions under which an SCI sample must be collected when the results will be submitted to DEP for assessment purposes. These procedures are intended to reduce the likelihood of a sample failing due to natural hydrological processes.

Lab Audit Findings

On January 7, 2019, DEP auditors visited McGlynn Laboratories to observe the processing of an SCI sample that was collected by McGlynn Laboratories on September 25, 2018 during the DEP Bioassessment Training event. The deficiencies listed below are numerous and systemic and demonstrate a critical lack of adherence to DEP SOPs.

SCI 1110. – *Stream Condition Index Sampling Records*
Section 2. - Sampling containers were not properly labeled, and there was no record of the preservative used. Insufficient sampling containers were available on the day the sample was collected. Material from the minor sweeps (*i.e.* sand) was placed into a labeled sampling container but the remaining material was in an unlabeled cooler.

SCI 2100. – *Stream Condition Index (SCI) Determination*

Section 3.1.1.1. – Some of the required equipment and supplies were not available or in use during the laboratory audit. Several items were in not use until the DEP auditor asked about them, including a

properly-sized gridded pan, a random number table, or a lighted-magnifier. The light that was used after DEP inquired was a halogen shop light that produced a significant amount of heat. The sorter became uncomfortable and turned the light off and continued to sort without it. During sample preparation, proper safety equipment was not in use. The analyst did not use a respirator, lab coat, or safety glasses. There was laboratory ventilation, but it was either insufficient or the rinsing was incomplete as the material caused the DEP auditor's eyes to water. The smell of formalin was noticeable.

Section 3.1.2.2. – The material collected during the sampling event was not homogenized. The sand was stored and processed separately from the rest of the sample material.

Section 3.1.2.3. – The material was initially placed directly into a bucket. It was not placed in the sieve until a DEP auditor asked about it. Dr. McGlynn did not know what size sieve was required until told by the DEP auditor. The material was not carefully placed into the sieve, and some of the material was spilled onto the outside of the sampling container, into the sink, or on the floor before it was inspected. Waste formalin was not collected into a container but was discarded directly down the drain until it was mentioned by a DEP auditor.

Section 3.1.2.4. After rinsing, larger pieces of material were placed into a plastic container for inspection by a second analyst. The analyst placed a large amount of material into the tray making it extremely difficult to thoroughly inspect the detritus. The analyst did not use magnification or a light source until it was mentioned by DEP; the light source was a shop light with a halogen bulb. After a short time, it was turned off as it was getting uncomfortably hot.

Section 3.1.2.5. Some of the gridded trays did not conform to DEP SOPs. The individual grids were raised on the outside and varied in size, and there were 15 larger grids, not 24 as instructed in the SOP. This was mentioned by the DEP auditors, and Dr. McGlynn brought out two glass trays on which 24 – 5 cm grids were drawn. The third tray was a different size (approximately 8 inches by 8 inches) and 16 grids were drawn onto the bottom. The grids in this third tray were 5 cm each and would likely have resulted in an exceedance of the target count. The SOP does not specify the size of grids in this third tray. It only states that they should be sufficiently small to achieve the target number of individuals. (The DEP Central Laboratory typically divides the third tray into 16-1 cm grids.)

At this point in the sample processing, it was clear that the DEP SOPs were not being followed. In the time remaining, DEP auditors demonstrated proper homogenization and the systematic scanning of sorted material for the organisms that would ultimately be included in the SCI sample.

Data Usability Recommendation

Based on the results of this audit, the DEP Aquatic Ecology and Quality Assurance Section has determined that the SCI data produced by McGlynn Labs do not meet the department's data quality objectives and recommends that the data not be used in support of the City of Marianna Wastewater Treatment Plant (WWTP) biennial monitoring required by permit (FLA101141-008-DW1P/NRL), in support of any other permit renewals, or for biological assessment under Chapter 62-303, F.A.C. The usability of the audited data for other purposes must be determined separately according to applicable requirements in rule 62-160.670, F.A.C.

Table 1. Audit findings for the Laboratory Records Audit of selected SCI sample data produced by McGlynn Labs, and DEP conclusions based on lab visit in January 5, 2019.

Finding	SOP Reference	Audit Finding (June 2018)	McGlynn Labs Response (August 2018)	DEP Comments and Required Corrective Actions (September 2018)	DEP Conclusion
1	SCI 2100 Section 3.1.2.4- 3.1.2.9	Gridded trays were not used. It is unclear how the material was separated into the two replicates to ensure randomization of organisms.	MLI will use 24 square gridded trays according to DEP-SOP-001/01; Biology SOP IZ-01 to IZ-04, IZ-06 to IZ-08, IZ-39 and Quality Control: Biology SOP IZ-13, IZ-40, BG-01, and BG-03.	The IZ and BG SOPs cited in McGlynn Labs' responses are internal DEP documents specific to its laboratory and cannot be used as official references. Entities submitting SCI data to DEP must follow SCI 1000 (Rev. Date: January 2017). The gridded trays specified in SCI 2200 Section 1.1 and 2.1 ensures that all organisms are thoroughly mixed throughout the detrital matrix and have an equal chance of being represented in the final sample. **Required**: Thoroughly mix and spread the detrital matrix evenly and systematically so that each organism has an equal chance of being included in the subsample without exceeding the target number of individuals.	During the Laboratory audit, DEP confirmed that the gridded trays were not being used properly. The grids in the tray initially shown were not of a correct or uniform size.
2	SCI 2100 Section 2.1- 2.2	It is unclear how McGlynn Labs achieved the target number of individuals from the documentation provided.	With a 6-month sampling window in the revised permit we will use 24 square gridded trays according to DEP-SOP-001/01; Biology SOP IZ-01 to IZ-04, IZ-06 to IZ-08, IZ-39 and Quality Control: Biology SOP IZ-13, IZ-40, BG-01, and BG-03	The IZ and BG SOPs cited in McGlynn Labs' responses are internal DEP documents specific to its laboratory and cannot be used as official references. Entities submitting SCI data to DEP must follow SCI 1000 (Rev. Date: January 2017). Systematically subsampling from the gridded trays as specified in SCI 2200 Section 2.1 and 2.2 ensures that the target number of individuals are achieved without being exceeded.	During the Laboratory audit, DEP confirmed that homogenization and subsampling protocols were not being done properly. DEP was unable to verify that McGlynn Labs can produce a representative SCI sample based on subsampling of a target number of individuals from fully homogenized material.

Finding	SOP Reference	Audit Finding (June 2018)	McGlynn Labs Response (August 2018)	DEP Comments and Required Corrective Actions (September 2018)	DEP Conclusion
				Required: Spread the detrital matrix evenly and systematically in a measured tray to ensure the target number of individuals is achieved without being exceeded.	
3	SCI 2100 Section 2.3	Dr. McGlynn does not report an "X" when there are too few organisms for a valid test.	In our reports, particularly the 2014 report results are stated as SW-1 (no SCI due wash out); SW-2 (valid SCI sample); SW-3 (no SCI due to drought conditions); SW-4 (no SCI due to drought conditions); SW-5 (valid SCI sample); SW-6 (no SCI due to drought conditions). In the future we will use an 'x.'	**Required**: Use an 'X' qualifier code when there are too few organisms for a valid SCI sample.	DEP was unable to verify that it was the site conditions and not deficiencies in sampling or sample processing that resulted in the samples having too few organisms for a valid SCI sample.
4	SCI 2100 Section 2.9-3.7	Dr. McGlynn had no records of his sorting protocols and does not track his sorting efficiency. Thus, compliance with the sorting efficiency guidelines could not be determined.	MLI will strictly follow and adhere to sorting protocols will be according to DEP-SOP-001/01; Biology SOP IZ-01 to IZ-04, IZ-06 to IZ-08, IZ-39	The IZ and BG SOPs cited in McGlynn Labs' responses are internal DEP documents specific to its laboratory and cannot be used as official references. Entities submitting SCI data to DEP must follow SCI 1000 (Revision Date: January 2017). **Required**: Track sorting efficiencies for each analyst.	During the Laboratory audit, DEP confirmed that homogenization and subsampling protocols were not being done properly. The SCI SOP states that the entire sample should be included in the reduction and homogenization process. DEP auditors noted that the sand was processed completely separately from the remaining material. The next steps involved gently rinsing the sample material and carefully emptying the contents into a sieve making sure no material was lost. DEP observed material being dropped into the sink, onto the floor, and remaining on the sides of containers. After rinsing, large pieces of material should be placed into a gridded pan and then systematically inspected under a lighted magnifier for organisms not removed during the rinsing process. The material

Finding	SOP Reference	Audit Finding (June 2018)	McGlynn Labs Response (August 2018)	DEP Comments and Required Corrective Actions (September 2018)	DEP Conclusion
					was not placed into a gridded pan and a lighted magnifier was not used until mentioned by DEP auditors. Dr. McGlynn indicated he intended to perform a check of this material and that this action would represent his quality control procedure of the sorting process. This step, however, only reflects a very small portion of the subsampling process and does not include checks of the actual sample aliquots, which should be the main focus of the sorting quality control measures.
5	SCI 2100 Section 3.1.2.10 and 2210 Section 1.2-1.5	Dr. McGlynn performs QC checks on his own samples. He has no procedures for establishing corrective actions should his sorting efficiency drop below the specified targets.	MLI will follow, diligently, DEP Quality Control: Biology SOP IZ-13, IZ-40, BG-01, and BG-0.	The IZ and BG SOPs cited in McGlynn Labs' responses are internal DEP documents specific to its laboratory and cannot be used as official references. Entities submitting SCI data to DEP must follow SCI 1000 (Revision Date: January 2017). **Required**: Have a second analyst randomly select one in every ten petri dishes for a QC check, as required by SCI 2100 Section 3.1.2.10 and SCI 2210 Sections 1.2–1.5. Track sorting efficiencies based on the results of these QC checks.	Although McGlynn Labs obtained a second sorting analyst, the overall sorting and sorting quality control measures were still inadequate to ensure that a valid and representative SCI sample result could be produced. A systemic lack of understanding of the sample preparation procedures was noted during the laboratory audit.
6	SCI 2220 Section 4.1	*Rhagovelia* sp. should have been collapsed into *R. obesa* and *R. torreyana* proportionately rather than being counted	MLI will collapse *Rhagovelia* sp. into *R. obesa* and *R. torreyana* with our taxonomist, Dr. John Epler.	**Required**: Follow collapsing protocols per DEP SOP SCI 1000 Section 2220, Section 4.1 (Rev Date: January 2017).	This response is acceptable and considered complete as of August 2018.

Finding	SOP Reference	Audit Finding (June 2018)	McGlynn Labs Response (August 2018)	DEP Comments and Required Corrective Actions (September 2018)	DEP Conclusion
		as an additional taxon.			
7	SCI 2100 Section 5.5	The metric calculations were based on an old version of the SCI (2007) rather than the current version (2012).	Metric calculations were updated from an old version of the SCI (2007) to the current version, DEP-SOP-002/01, LT 7000 Determination of Biological Indices, and Revision Date: January 2017, particularly LT 7040.	SCI Index calculations are no longer in the LT series. The current version is in SCI 2100 Section 5. **Required**: Follow the metric calculations as described in DEP SOP SCI 2220 Section 5.5 (Rev Date: January 2017).	This response is acceptable and considered complete as of August 2018.
8	SCI 2220 Section 5.1-5.5	There were multiple errors in the assignment of taxa to the various metrics as well as individual counting errors.	MLI will refine assignment of taxa to the various metrics with our taxonomist, Dr. John Epler.	**Required**: Use the established lists of taxa when assigning taxa to the various metrics.	This response is acceptable and considered complete as of August 2018.
9	SCI 2100 Section 5.9	Metrics were not rounded down to 10 when the raw calculations resulted in values >10.	MLI will correct these calculations, metrics will be rounded down to 10 when the raw calculations result in values >10.	**Required**: Round down each metric to 10 when the raw calculations result in values greater than 10.	This response is acceptable and considered complete as of August 2018.
10	SCI 2100 Section 5.11	Individual replicate sums were not divided by 0.9.	MLI will correct this calculation, individual replicate sums will be divided by 0.9.	**Required**: Individual replicate sums must be divided by 0.9.	This response is acceptable and considered complete as of August 2018.
11	SCI 2100 Section 5.12	Individual replicate scores were not averaged into a single final SCI score. All errors combined resulted in differences in 10 points for SWD-02 and 9 points for SWD-04.	MLI will average individual replicate scores into a single final SCI score.	**Required**: Average each individual replicate score into a single final SCI score. Report the final score as a whole number.	This response is acceptable and considered complete as of August 2018.
12	SCI 2230 Table SCI 2230-1	*Dolomedes* sp. is considered a semi-aquatic organism and is not listed in Table	*Dolomedes* sp. will never again be included in the taxa count.	**Required**: Only include taxa listed in Table SCI-2230-1 in the SCI metric calculations.	This response is acceptable and considered complete as of August 2018.

Finding	SOP Reference	Audit Finding (June 2018)	McGlynn Labs Response (August 2018)	DEP Comments and Required Corrective Actions (September 2018)	DEP Conclusion
		SCI-2230-1. It should not have been included in the taxa count.			