Stream Condition Index (SCI)

Sample Preparation

1. SCOPE AND APPLICATION

1.1 This method details the rinsing, sorting, and storing of macroinvertebrate samples collected with a #30 mesh D-frame dipnet.

1.2 This method is used for samples collected per DEP Standard Operating Procedure (SOP) SCI 1000 – *Stream Condition Index Methods* in streams deemed to have acceptable conditions for calculating the Stream Condition Index (SCI) per SOP SCI 2000 – *Stream Macroinvertebrate Laboratory Procedures and Index Determination*

1.3 This method is to be used for all samples with Laboratory Information Management System (LIMS) test code MI-FW-QLDC.

2. SUMMARY OF METHOD

2.1 A biological sample, consisting of surface water, leaves, stems, sand, silt and other detritus with formalin as a preservative, is emptied into a U.S. #30 mesh sieve over a discard bucket to catch the waste formalin and all material smaller than 30-mesh.

2.2 The sample is then placed into a bucket of water in order to homogenize the sample. Floating material and macroinvertebrates are again poured through and retained in the 30-mesh sieve.

2.3 After all floatable material has been removed, large material, such as rocks, twigs, and leaves, is then removed and washed over the 30-mesh sieve until the material remaining in the bucket is mostly shell hash and/or sand.

2.4 The large material, shell hash, and sand are examined under a magnifying lamp to find any remaining organisms. Any organisms found in this step are added to the material in the 30-mesh sieve. The sieve is placed in a pan containing enough water to cover the material on the screen and gently agitated to remove any remaining particles and material smaller than 30 mesh.

2.5 The material remaining after sample rinsing is transferred to a gridded tray where sections are randomly removed and placed into a second gridded tray. Sections are then randomly removed from the second tray and placed into a third, small gridded tray which is divided into 16ths.

2.6 The material in the small gridded tray is placed into a petri dish 1/16 at a time. This material is examined under a dissecting microscope to search for macroinvertebrates. Organisms are transferred to an ethanol-filled glass vial. Material is examined in 1/16 portions until a target of 140-160 macroinvertebrates is reached. Once the target is reached, another subsample from the first gridded tray is processed in the same manner to create a replicate. The macroinvertebrates in replicate 1 and 2 are kept in separate vials for identification.

3. APPARATUS AND EQUIPMENT

3.1 Waterproof paper and pencil
3.2 Fine tip permanent black marker
3.3 U.S. #30 mesh sieve

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3.4 U.S. 5/16-inch sieve
3.5 Large diameter funnel
3.6 Bucket
3.7 Shallow pan
3.8 O.C. White ® lighted magnifier
3.9 3 trays marked with a grid of 24 equal-sized, 5 cm squares
3.10 1 tray marked with 16 equal-sized squares
3.11 List of random numbers or an approved digital random number generator, such as randomizer.org
3.12 4 jars (250 mL)
3.13 Dissecting microscope
3.14 Petri dishes (100 x 15 mm), 10 per sample
3.15 Forceps
3.16 2 vials for picked organisms (1 or 2 dram)
3.17 Laboratory counter
3.18 Squeeze bottle of 80% Ethanol
3.19 Safety equipment (see section 9.1)
3.20 Kimtech™ wipes
3.21 Ethanol waste bucket/container
3.21 Funnel (to filter waste)
3.22 Squeeze bottle of Ethanol/Glycerin

4. REAGENTS AND CHEMICALS

4.1 Ethanol (80%) - prepared following Standard Operating Procedure (SOP) IZ-09
4.2 Ethanol/Glycerin mixture - Fill a 500 mL squeeze bottle with 20 mL of pure Glycerin. Add 80% Ethanol (prepared according to IZ-09) up to the marked fill line and replace the cap. Place one finger over the nozzle and gently shake the bottle to mix. Label appropriately.

Note: The purpose of the ethanol/glycerin mixture is to ensure samples stay hydrated when not being actively worked on. It is not necessary for the measurements to be exact.

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab per SCI 1000 – *Stream Condition Index Methods*.

5.2 Samples are checked into the laboratory per SOP IZ-01.

6. SAMPLE PREPARATION PROCEDURE

6.1 Select a sample. If two or more containers are taped together, check that all containers are part of the same sample.

6.1.1 Make sure you include all containers in which the particular sample is stored (there may be several jugs). The entire sample must be included in this reduction and homogenization process.

6.1.2 If two different samples are accidentally taped together, there is no way to separate them once homogenization occurs. Both samples will be lost.

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6.2 Check out the sample using the Laboratory/Receiving/Custody utility in the LIMS. Create a new prep worksheet using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS.

6.3 Using the Laboratory/Receiving/Print Preparation Labels utility in LIMS, print out 10 labels for dishes, 12 vial/tray labels, and a bench sheet. All labels are printed from the Sato printer.

6.3.1 To print labels, go to the sample Log-in station, open LIMS and select "Laboratory," "Receiving," "Print Preparation Labels." To print dish labels, go to "Invert Labels," "Labels for Dishes," scan the sample bar code, select "File," "Print," select the "EAR-Labs-LBL19" printer, and print 10 copies. To print vial and jar labels, select "Invert Labels," "Vial Labels Rep 1," and print 6 copies. Repeat this process for Rep 2 vial labels. For Tray labels, use vial labels with an appropriate Rep #. For Trays 0 and 3, use a fine-point permanent marker to rename two vial labels. To print a bench sheet, select bullet next to "Sample ID," scan sample, go to "Bench sheets," "Macroinvertebrate," select "File," "Print," select the "LAB" printer, print 1 copy. (The lab printer is a Brother printer. If it is not working you can use the PRN1 printer located in the office).

6.4 Using waterproof paper and pencil make clearly written labels to go inside the 2 vials of picked bugs. The labels should include: LIMS sample ID#, date sampled, and whether it is Replicate 1 or 2. For each replicate, place the waterproof label inside the vial and the corresponding vial label on the outside of the vial. Using permanent marker, label the lid with the same information. Check to ensure that all label information on each vial represents the same replicate number.

6.5 Put on the proper safety equipment. Place a U.S. #30 mesh sieve over a discard bucket. Empty the contents of the sample container into the sieve slowly, making sure not to lose any of the sample. The formalin will go through the sieve and collect in the bucket. After it has finished draining, pour the waste formalin into the proper container.

6.5.1 Material from all of the sample containers may not fit in the sieve at the same time. If this is the case, put the material in the sieve in batches and then combine in a bucket.

6.5.2 Formalin disposal containers are located under the sinks in the main laboratory (B205).

6.6 Ensure all organisms and material has been transferred to the sieve. Do not remove the labels from the sample containers. Clean the containers and place them to dry. Sample bottles are reused after cleaning and LIMS discard. Check the sample in using the Laboratory/Receiving/Custody utility in the LIMS.

6.6.1 The LIMS Custody utility is tracking the sample bottle, not the contents. Empty containers will be kept for a period of time, designated by the Lab Data Support (LDS) staff, after the job is authorized in LIMS. After this period of time, the invert group discards sample containers from LIMS and stores the empty bottles in a designated location on the fourth floor. The Receiving staff will collect the empty bottles for re-use.

6.7 Rinse the entire sample with water a few times to remove any remaining formalin from the sample. Place the entire sample into a bucket and fill the bucket about 1/2– 2/3 with water. Gently stir the mixture and then pour through the U.S.5/16 inch sieve sitting on top of the #30 mesh sieve to separate floating debris. Repeat this process until most the floatable material is removed from the sample.

6.7.1 With the change of formalin to water, many of the small organic particles (including macroinvertebrates) will float to the surface.

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6.8 Begin removing some of the large material (leaves, twigs, rocks, etc.) by continuously picking large pieces out of the 5/16 sieve and washing them over the bucket or sieve. Do not discard this material; place it into a pan. When the bucket is full of water, pour the elutriate through the U.S. 5/16 sieve and the #30 mesh sieve. Keep repeating until you have separated the light material from the heavier material (i.e. light organic matter from coarse sand). This process is repeated until only the coarse material (i.e. shell hash and/or sand) is left in the bucket. Rinse the course material into a pan.

6.8.1 Gently rinse the sample material with tap water making sure the water pressure does not damage the organisms (a small hose attached to the faucet works best).

6.8.2 It has been shown that 80 to 95% of the individuals in the sample will be found in the elutriate.

6.8.3 In the end there will be a pan full of leaves, twigs, rocks, etc., a pan full of sand, shell hash, etc., and the organic detritus that is in the #30 mesh sieve.

6.9 When the rinsing process is complete, there will be three portions of the sample. The first is the large material that was picked out of the sample. The second will be primarily coarse sand. Examine these two portions under the lighted magnifier for organisms that weren't removed by the rinsing process. If any organisms are found, randomly place them into the third and final portion; the elutriate material in the # 30 mesh sieve, which will contain the majority of the organisms. Have another analyst scan through the material in each pan before discarding. Any organisms found by the second analyst should be added to the sieve.

6.9.1 This inspection is best accomplished by placing the debris in a pan with enough water to cover the material and then observing it with the O.C. White lighted magnifier.

6.9.2 Use a waterproof label with the name of the person sorting the sample and "Please QC" on opposite sides of the paper for each tray. Alternatively, if the Trays will be exposed for a period greater than a day, cover them and the remainder of the sample with foil. Use a permanent marker to label the foil with sample ID, sorter's initials, and date covered. To keep the contents of the #30 mesh sieve hydrated, it should be placed into a pan and filled with water as in 6.10 before being covered. With many different trays, this helps prevent confusion regarding which step in the process the trays are in.

6.10 Place the U.S. #30 mesh sieve containing elutriate into a pan. Fill the pan with enough water to cover the top of the sieve screen. With the appropriate up and down and back and forth motion, most of the very small particles will pass through the sieve into the tray. Do this for approximately 2 minutes and then discard the water. Repeat the procedure until the water remains clear or the process has been done five times, whichever is first. This step also helps distribute individuals evenly in the sieve.

6.11 Place the sieved sample into a gridded tray (Tray 0). Each 5 cm grid should have a pre-assigned number, 1 through 24. Place the Tray 0 sticker on Tray 0.

6.11.1 The sample may not cover all 24 grids. It should be spread out evenly across a number of grids that is divisible by 3 (e.g., 12, 18, 21, etc.). Avoid smearing the material into the sides and corners of the trays.

6.12 Using the random number table or approved random number generator, select 1/3 of the total squares in Tray 0 (e.g., 8 of 24 squares) and spread those out as much as possible into a second, standard gridded tray (Tray 1). Place the Vial Rep 1 sticker, including analyst's initials, on Tray 1.

6.12.1 Use a ruler or other straight edge to delineate the edges of a grid while removing the sample.

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6.13 Using the random number table, select a square from Tray 1 and place it into a smaller tray (Tray 3), which has been subdivided evenly into 16ths. Place a Rep 1 vial sticker on Tray 3.

6.13.1 These small divisions help avoid going over the target number of individuals.

6.13.2 If the sample is particularly dirty, carefully place the Tray 1 square into a small U.S. #30 sieve and repeat step 6.10 to remove small particles before transferring the material to Tray 3.

6.14 Select 1/16 to begin the sorting process. Place the 1/16 into a Petri dish and add ethanol until the material moves around easily with forceps. Under a dissecting scope set at low power (approximately ranging from 7x to 50x), systematically scan back and forth or up and down picking every organism from the Petri dish and placing it into the appropriately labeled, ethanol-filled vial.

6.14.1 Using a pair of forceps in each hand enables you to better tease organisms out of fibrous detritus. Forceps should be sharp and properly aligned.

6.14.2 Use a laboratory counter to keep a running total of the number of organisms picked.

6.14.3 Drawing lines on the bottom of all ten Petri dishes will help in the systematic removal of organisms.

6.15 Continue this process until the target number of organisms is reached. The target number of organisms is 150 with an acceptable range of 140-160. Once a 1/16 or other subdivision is selected, its entire contents must be sorted.

6.15.1 Petri dishes should be saved until there are 10 or the target number of individuals has been reached. At that point another analyst should recheck one of the dishes. If organisms are found, the information is recorded, and the organisms are added to the sample vial and count toward the total number of individuals sorted. All dishes should be rechecked during training (see SOP IZ-40). After a dish has been checked, the ethanol in the remaining dishes is emptied into a waste container and the material may be discarded. During this process, a funnel and make-shift sieve using Kimtech™ wipes should be used to filter large solids from waste ethanol. The used wipes and the filtered solid material can be disposed of in a normal waste bin. The filtered waste ethanol can be disposed of in the approved waste disposal container.

6.15.2 The ethanol disposal container is located under the sinks in the main laboratory (B205).

6.15.3 Additional subdivision can be done at any point during the sorting. For example, a whole 16th may be sorted yielding 102 organisms, and then the next 16th could be subdivided into 2 parts to presumably yield 51 organisms for a total of 153 organisms.

6.15.4 If more than the target number of organisms is sorted, additional subsampling in SOP IZ-06 will reduce the number of organisms to be identified.

6.16 If the target count has not been reached with the entire contents of Tray 3, randomly select another square from Tray 1 using a random number table or approved random number generator and repeat steps 13-15 until the target number of organisms has been reached. Note that as before, once a 1/16 or other subdivision is selected, its entire contents must be sorted.

6.16.1 If the time taken to pick one replicate is nearing 12 hours, notify the Taxonomy Coordinator and Supervisor.

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6.16.2 If 1/3 of the squares in Tray 1 have been picked, and each square is consistently giving a low number of organisms such that the material will not yield the target number of organisms, notify the Taxonomy Coordinator and Supervisor.

6.17 If all of the material in Tray 1 has been sorted and the target number of individuals has not been reached, sorting has not exceeded 12 hours, and it is possible to get the target number of organisms from the material, go back to Tray 1 and repeat the entire process from Step 6.12.

6.18 Record the number of squares and subdivisions sorted. This information is necessary to calculate the portion of the fraction of the sample sorted.

6.18.1 Example: Subsampling data: 8/24 x 2/12 x 1.1875/2 =. Where 8 is 1/3 of the original 24 squares from Tray 0, 12 is the number of squares the 1/3 was spread across in Tray 1, 2 is the number of squares selected from Tray 1, and 1.1875 the amount of material sorted to obtain the target number of individuals (1 entire square from Tray 1 and an additional 3/16 from a second Tray 1 square).

6.18.2 Failure to record the subsampling data compromises the usefulness of the data.

6.18.3 After the target number of individuals has been reached, record at the top of the bench sheet the sorter, date of completion, and duration of this part of the analysis. Tally and record the number of individuals picked. Repeat for Rep 2.

6.19 Place a new Rep 1 vial sticker to a 250 mL jar and place the remaining 16^{ths} (from Tray 3) in the jar, making a note on the sticker of the amount of material in the jar (e.g. 4/16). Add your initials in case you need to store the sample in another location due to storage space.

6.19.1 After QC, discard any remaining material from the Petri dishes.

6.19.2 Occasionally, the actual number sorted is mistakenly lower than the target count; additional organisms can be sorted from this material in order to reach the target count. If more organisms are needed, update on the bench sheet the amount of material sorted and the extra time spent.

6.20 Randomly select one additional square from Tray 1 for Replicate 1 and place it in a separate 250mL jar labeled with a Rep 1 vial sticker.

6.20.1 Occasionally, the actual number sorted is mistakenly lower than the target count; additional organisms can be sorted from this material in order to reach the target count. If more organisms are needed, update on the bench sheet the amount of material sorted and the extra time spent.

6.21 Going back to Tray 0, repeat the entire process from Steps 6.12 – 6.20 to obtain a second 150-count for Replicate 2, placing the new squares into the third 24 division Tray, labeled Tray 2. Label as appropriate for Tray 2 instead of Tray 1. Tape the vials for Rep 1 and Rep 2 together.

6.21.1 If the target number of individuals for both replicates cannot be met, notify the Taxonomy Coordinator and Supervisor. Before the sample is given to the taxonomists, the Coordinator or Supervisor will notify the customer and try to identify the cause for the low number of organisms.

6.21.2 If the sample is taking more than three days to pick, hydrate the sample with 80% Ethanol/Glycerin mixture. Make sure to hydrate the entire sample in all of its trays.

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6.22 Using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS, complete the prep worksheet. Record completion of preparation and QC on the Invertebrate Groups Cummulative Sort QC excel document located at "X:\Labs\Lab5\Biology\InvertGroup\Sample_Prep\Cummulative_Sort_QC_March_2024.xlsx".

7. SAMPLE ANALYSIS PROCEDURE

7.1 Samples are analyzed per SOP IZ-06.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all bench sheet and form entries.

8.2 Department of Environmental Protection (DEP) Laboratory Macroinvertebrate Bench sheet

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

8.4 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.

8.5 QC of Sorting is performed per SOP IZ-40.

8.6 QC of Taxonomic Identification is performed per SOP IZ-13.

8.7 Refer to the Laboratory Quality Manual.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1 Lab coat, latex or vinyl gloves, and eye protection are required when working with formalin.

9.2 Avoid breathing formalin fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well-ventilated area.

9.3 When rinsing samples preserved with formalin, catch the "first flush" and transfer the waste formalin into a hazardous waste storage container.

9.3.1 Formalin disposal containers are located under the sinks in the main laboratory (B205).

9.3.2 Handling of waste formalin is performed per SOP IZ-10 and SOP IZ-38.

9.4 When discarding material from petri dishes, collect and transfer the waste ethanol into a hazardous waste storage container.

9.4.1 The ethanol disposal container is located under the sinks in the main laboratory (B205).

9.5 Squeeze bottles must be labeled with the name and concentration of the reagent.

9.6 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

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9.7 Refer to the Laboratory Health and Safety Plan.

10. REFERENCES

10.1 DEP Laboratory Quality Assurance Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

10.2 DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

10.3 SOP SCI-1000, *Stream Condition Index Methods*

10.4 SOP IZ-01, *Sample Arrival/Log-in Procedures*

10.5 SOP IZ-06, *Invertebrate Enumeration and Taxonomic Analysis*

10.6 SOP IZ-09, *Preparation of 80% Ethanol*

10.7 SOP IZ-10, *Preparation and Proper Disposal of Buffered Formalin*

10.8 SOP IZ-13, *Invertebrate Taxonomic Identification Quality Control*

10.9 SOP IZ-38, *Formalin Assay, Recycling and Buffering*

10.10 SOP IZ-40, *Invertebrate Sorting Quality Control (QC)*

10.11 SOP LB-002, *Non-conformance Reporting System*

Appendix of Significant Changes

12/01/2009	Reformatted the layout following SOP LB-001.
12/01/2009	Added extra quality assurance check (6.1, 6.1.2); modified the creation and completion of the prep worksheet in LIMS (6.2 and 6.22).
12/01/2009	Added changes in use of the LIMS custody utility (6.6) and rinsing to step 6.7 and labeling of trays to step 6.9.
12/01/2009	Added an additional sieving to step 6.13 for randomly chosen individual squares.
12/01/2009	Added circumstances for which to notify Supervisor and Manager (6.16.1, 6.16.2, 6.21.1) and a directive to update sorting documentation (6.19.2, 6.20.1).
12/01/2009	Moved what is now 6.15.3 and 6.15.4 from 6.16.
11/10/2011	Added ethanol handling and disposal to section 9. Updated name for the Laboratory Safety Manual.
11/04/2013	Removed formalin respirator requirement. Updated sorting QC and formalin SOP #s.
10/27/2014	Formatted for 508 compliance
11/05/2015	Updated SOP references. Included reference to form IZ-LOG.
11/19/2015	Removed reference to the Log In logbook and corrected form name to IZ-SORT.
12/30/2016	Standardized SOP naming conventions; Added note that sample bottles are reused. Removed reference to the Log In logbook.

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01/29/2019	Added instructions for filling out the bench sheet.
01/15/2020	Removed the word sieved in section 2.2, updated discard information section 6.6.1 receiving does not do process Todd does, updated section 6.19 add initials to jars when storing.
12/03/2021	Updated, label printer name and broken link to Health and Safety Plan.
11/14/2022	No changes.
11/08/2023	No changes.
110/8/2024	Revised the Tray System to account for now using Tray ‘0’ and labeling both reps as Trays 2 and 3. Revised label printing instructions for methods currently in use. Added specific instructions for filtering waste Ethanol. Added instructions to hydrate the sample. Revised the QC collection document from IZ-SORT-(version #) to the Invert groups shared Excel document.

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