Invertebrate Core/Grab/Dredge Sample Preparation

(Modified from *Standard Methods 10500C*)

1. SCOPE AND APPLICATION

1.1 This method details the rinsing, sorting, and storing of core and grab/dredge samples collected with a coring device, a petite ponar, or a vanveen.

1.2 This method is used for samples collected per DEP Standard Operating Procedure (SOP) FS 7000 – *General Biological Community Sampling* in environments deemed to have acceptable conditions for calculating macroinvertebrate community and diversity measures per SOP LT 7000 – *Biological Indices*.

1.3 This method is to be used for all samples with Laboratory Information Management System (LIMS) test codes ending in QNCC, QNCP, 3COR, QNNC, VNUK, or VNSG.

1.4 This Standard Operating Procedure (SOP) incorporates previous versions of SOP IZ-05, *Invertebrate Grab/Dredge Sample Preparation*.

2. SUMMARY OF METHOD

2.1 A biological sample, consisting of surface water, macroinvertebrates, 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 sieved sample is placed into a bucket of water 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 examined under a dissecting microscope to search for macroinvertebrates. There is no subsampling performed on these samples. All organisms are transferred to an ethanol-filled glass vial for identification.

3. APPARATUS AND EQUIPMENT

3.1 Waterproof paper, pencil, and black pen
3.2 Fine tip permanent black marker
3.3 U.S. #30 mesh sieve
3.4 U.S. 5/16-inch sieve
3.5 Large diameter funnel

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3.6 Bucket
3.7 Shallow pan
3.8 Luxo® lighted magnifier
3.9 Glass jars, 100 to 250 mL
3.10 Dissecting microscope
3.11 Petri dishes (100 x 15 mm), 10 per sample
3.12 Forceps
3.13 Vial for picked organisms (1 or 2 dram)
3.14 Laboratory counter
3.15 Squeeze bottle of 80% Ethanol
3.16 Safety equipment (see section 9.1)

4. REAGENTS AND CHEMICALS

4.1 Ethanol (80%) – prepared following SOP IZ-09

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab per SOP FS 7000.

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 know how many containers in which the 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.

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 dish labels, 2 vial labels, and the appropriate 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 “Generic / Text only ” printer, print 10 copies. To print vial and jar labels, select “Invert_Labels”, “Vial Labels” and print 2 copies. To print a bench sheet, select bullet next to “Sample ID”, scan sample, go to “BenchSheets”, select the appropriate

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bench sheet from the list (either Freshwater or Marine Invertebrate), select “File”, “Print”, select the “LABB2_20” printer, print 1 copy.

6.4 Using waterproof paper and pencil make a clearly written label to go inside the vial of picked bugs. The label should include: LIMS sample ID#, project, station identification, and date sampled. Place the waterproof label inside the vial and the vial label on the outside of the vial. Using a permanent black marker, label the lid with the same information.

6.5 Put on the proper safety equipment. Place a U.S. 5/16-inch sieve on top of a U.S. #30 mesh sieve and place together 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 the sample has finished draining, pour the waste formalin into the proper waste container.

6.5.1 Material from all 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 (see step 6.7).

6.5.2 Formalin waste 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. Clean the containers and place them to dry. 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 Receiving staff will collect the empty bottles and discard them from LIMS.

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 sieve to separate floating debris. Repeat this process until most of the floatable material is removed from the sample.

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

6.8 Begin removing some of the large material (leaves, twigs, rocks, etc.) by continuously picking large pieces out and washing them over the bucket or sieve. Do not discard this material, but place it into a pan filled with enough water to cover the material. When the bucket is full of water, pour the elutriate through the U.S. #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.

6.6.1 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).

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

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6.6.3 You should end up with a pan full of leaves, twigs, rocks, etc. and a bucket full of sand, shell hash, etc.

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, rinsed, and placed in a pan. The second is the coarse sand in the bucket. Transfer the sand to a separate pan filled with enough water to cover the material. 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 material in the sieve, which contains the majority of 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 Luxo® 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. 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 the material 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 you have done the process five times, whichever is first. This step also helps distribute individuals evenly in the sieve.

6.11 Using an ethanol-filled (80% ethanol) squeeze bottle, rinse the organisms plus detritus matrix from the sieve into the smallest practical container (usually a 100-mL to 250-mL glass jar). Place one printed vial label with the sample information on the jar.

6.12 Place a small amount of detritus plus organism mixture into a Petri dish and add ethanol until the material moves around easily with forceps.

6.12.1 Do not overload the petri dish with too much material, as this may result in organisms being overlooked.

6.12.2 The top portion of the petri dish may later be placed over the sample to prevent desiccation if you must leave the sample overnight.

6.13 Under a dissecting microscope set at low power (approximately 7x or 10x), systematically scan back and forth or up and down, picking every organism from the Petri dish and placing it into an 80% ethanol-filled vial.

6.13.1 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.

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

6.13.3 Drawing lines on the bottom of the Petri dish will help in the systematic removal of organisms.

6.14 Continue this process until the sample is finished.

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6.14.1 Petri dishes should be saved until you have 10; 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. After a dish has been checked, the ethanol in the remaining dishes is emptied into a collection container and the material may be discarded. All dishes should be rechecked during training (see SOP IZ-40).

6.15 Using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS, complete the prep worksheet. Record completion of preparation and QC on the form IZ-SORT-(version #), *Macroinvertebrate Sample Sorting*.

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 Macroinvertebrate Sample Sorting form

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.

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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.

9.7 Refer to the Laboratory Health and Safety Plan.

10. REFERENCES

10.1 DEP Laboratory Quality 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.fldepnet.org/content/dear/bureau-laboratories-safety-documents

10.3 Form IZ-SORT-(version #), *Macroinvertebrate Sample Sorting*

10.4 SOP FS-7000, *General Biological Community Sampling*

10.5 SOP LT-7000, *Biological Indices*

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

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

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

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

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

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

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

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

Appendix of Changes

12/01/2009 Reformatted the layout following SOP LB-001, added extra quality assurance checks (6.1, 6.1.1, and 6.1.2), modified the creation and completion of the prep worksheet in LIMS (6.2 and 6.15.1), added changes in use of the LIMS custody utility (6.6) and moved rinsing to step 6.7.

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

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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.
02/22/2017	Standardized SOP Naming Conventions.
03/13/2018	Updated DEP Laboratory Quality Manual link

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