Invertebrate Enumeration and Taxonomic Analysis

(Modified from Standard Methods 10500C)

1. SCOPE AND APPLICATION

1.1 This method details the counting and identification of aquatic macroinvertebrate samples collected according to DEP Standard Operating Procedures (SOPs).

1.2 This method applies to macroinvertebrate samples with Laboratory Information Management System (LIMS) test codes beginning in MI-.

2. SUMMARY OF METHOD

Sorted samples of aquatic macroinvertebrates are emptied into a petri dish or Syracuse watch glass. Some may need to be mounted onto slides. The identification, to the lowest practical taxonomic level, and enumeration of each organism is recorded on a bench sheet. Only organisms with heads are identified and counted. Taxonomic data is entered into the State Biological Database (SBIO) and the sample result into LIMS.

3. APPARATUS AND EQUIPMENT

3.1 Dissecting and compound microscope
3.2 Identification References
3.3 Squeeze bottle of 80% Ethanol
3.4 Forceps
3.5 Petri dishes (100 x 15mm or 60 x 15mm) or Syracuse watch glass (2-5/8” diameter)
3.6 Laboratory counter (optional)
3.7 Random numbers table

4. REAGENTS AND CHEMICALS

4.1 Ethanol (80%) - prepared following Standard Operating Procedure (SOP) IZ-09

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab according to FS 7000 – *General Biological Community Sampling*, SCI 1000 – *Stream Condition Index Methods*, or BRN 1000 – *Biological Reconnaissance Field Methods*.

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

6. SAMPLE PREPARATION PROCEDURE

6.1 Samples are prepared according to SOP IZ-02, IZ-03, IZ-04, or IZ-39, depending on the type of sample.

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7. SAMPLE ANALYSIS PROCEDURE

7.1 Select a sample and find the matching bench sheet that includes LIMS sample #, site description, project, and date collected.

7.2 Depending on the type of sample being identified, the proportion of the original sample to be identified is different.

- For marine core and Ponar samples, all organisms in the entire sample are identified. There is no subsampling done for marine samples. Refers to sample test codes MI-MN-QNCC, MI-MN-QNCP, MI-MN-3COR, MI-MN-QNNC, MI-MN-VNUK, or MI-MN-VNSG.
- For Hester Dendy and freshwater Ponar samples, all organisms in the entire sample are identified except for worms and midges, which are each subsampled to a target count of 100-110 following SOP IZ-07 and mounted following SOP IZ-08. Refers to sample test codes MI-FW-QNAC and MI-FW-QNNC.
- For samples on which the Stream Condition Index (SCI) will be calculated, the original sample material was subsampled following SOP IZ-02 to obtain 140-160 organisms for identification. Refers to sample test code MI-FW-QLDC.
- For Lake Condition Index (LCI) and Everglades samples, the original sample material was subsampled following SOP IZ-39 to obtain 100-110 organisms for identification. Refers to sample test codes MI-FW-QLNC and MI-EV-QLDC.

Note: Some marine core, marine Ponar, Hester Dendy, and freshwater Ponar samples may contain close to a thousand individuals of a single, dominant taxon. For these rare instances, these sample types could be subsampled, either before or after sorting, using a box subsampler, a Folsom splitter, or the USGS method of quartering the sample in a tray or sieve. The same method must be used for all samples or replicates from a site, event or project. The subsampling factor is applied to the results to estimate the taxonomic content of the entire sample.

7.3 If the entire sample is to be identified or the target number of organisms was sorted, identify the organisms following Steps 7.6-7.11. Occasionally, during the sample sorting process, more or fewer than the target number of organisms are sorted. If more than the target number of organisms were retained, further subsampling is required following Step 7.4. If fewer than the target number of organisms were retained, more organisms must be picked following Step 7.5.

7.4 If **more** than the target number of organisms were sorted, all organisms should be identified following Steps 7.6-7.11, but the organisms should be further subsampled after identification. Randomly select an individual and reduce the count by one from the bench sheet for each name selected. Repeat until the total number of individuals is within the target range of individuals. In the space provided at the bottom left corner of the bench sheet (in the "notes" section) record how many individuals were removed.

Note: One way to do this is to use a random number generator, like the one found at Randomizer.org or the RAND function in Excel, to create a list of random numbers. List the taxa names from the bench sheet onto a separate piece of paper or spreadsheet. The taxa should be listed to match the number identified. Example: There were 5 *Hyalella azteca* identified in the sample therefore *Hyalella azteca* should be listed 5 times. Alternatively, the order and counts already on the bench sheet can be used. Starting with the first random number generated, find the name on the ordered list that corresponds to this number and reduce the count by one on the bench sheet.

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7.5 If **fewer** than the target number of organisms were sorted, additional organisms should be sorted from the material retained during processing. Identify the additional organisms sorted following steps 7.6-7.11.

- For SCI samples, see SOP IZ-02.
- For LCI, Everglades dipnet, and other types of samples requiring subsampling, see SOP IZ-39.

7.6 Organisms are emptied from the sample vial into a Petri dish or Syracuse dish watch glass. Check the vial carefully to ensure no organisms are stuck to the sides of the vial or in the lid. Organisms other than worms, midges, and some aquatic mites are identified with a dissecting microscope.

After any necessary subsampling of worms and midges (see SOP IZ-07), slide mounts of worms and midges are made (see SOP IZ-08), (with one exception (see 7.6.1)), and identified using a compound microscope. Record the name, date and the duration of time for slide making on the bench sheet.

7.6.1 Wet identification of some of the midges is possible if both the following two criteria are true:

1) The taxonomist mounting the organisms is the same person who will be identifying the organisms.

2) That taxonomist has successfully completed a wet midge identification proficiency demonstration (per SOP IZ-13).

7.7 Identify organisms to the lowest practical taxonomic level using the most appropriate taxonomic reference for each group. Sometimes, parts of organisms (e.g., mayfly heads, etc.) must be mounted and examined using a compound microscope for proper identification. Make a note and indicate on the bench sheet next to the identification that parts of the organism were removed and mounted. Organisms are enumerated and, using forceps, are placed into a labeled vial containing 80% ethanol. Removing organisms from the Petri dish or Syracuse watch glass as they are counted will prevent double counting. Record, on the bench sheet, the identification and number of individuals for each taxon.

Note: Prior to identification, analysts may choose to separate the organisms into like groups. This can be done by placing the groups into smaller (60-mm diameter) individual Petri dishes or by grouping the organisms within the larger dish.

Note: After using a dichotomous key to arrive at the name of an unknown organism, it is important to check the organism's geographic range, habitat preferences, and morphological diagnosis to confirm that the identification is correct. Never ID an organism with pictures only and then assign a name based solely on a superficial resemblance.

Note: Do not identify Chironomid pupae beyond the level of family.

For a complete list of lowest practical level see Table LT 7900-1, http://publicfiles.dep.state.fl.us/dear/sas/sopdoc/2008sops/lt7000.pdf

Note: If an organism that can usually be identified to a lower taxonomic level is left at a higher taxonomic level, make a note of the reason on the bench sheet (i.e. too small; damaged; etc.)

Note: Use a laboratory counter to facilitate the enumeration process, especially where large numbers of individuals are present. Counters can be temporarily labeled to avoid mistakes. If a counter is not used, tally the number of each taxon on the bench sheet.

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Note: Any organism parts that are necessarily damaged or become loose should be retained and stored in the sample vial or in a micro vial within the vial, when possible.

Note: It will take some time until sufficient experience with identification procedures and references is gained. In addition to the ID manuals, the lab maintains a reference collection that can be used to compare specimens and facilitate identification.

7.8 After all wet taxa have been identified, record at the top of the bench sheet the identifier, date of completion, and duration of this part of the analysis. Tally and record the number of wet individuals identified and number of slides, if any.

7.9 If an organism is encountered in the laboratory for the first time or it is of interest, it should be kept for the reference collection or for verification. Remove the organism and place it in an individual vial containing 80% ethanol and labeled with county, location, sample #, date collected, taxon name, identifier, and date identified. Make a note of this on the bench sheet, so that it can be located in the future, if necessary.

7.10 For slide mounted organisms, write the taxon name directly on the slide label. If the specimen has no head or belongs to a group not classified as a benthic macroinvertebrate (e.g., nematode), draw a horizontal line on the coverslip to indicate that it will not be counted. Do not count specimens without heads. This situation is most common with worms.

7.11 Transcribe the individual taxa names and counts for the slide mounted organisms onto the bench sheet. Record at the top of the bench sheet the identifier, date of completion, and duration of this part of the analysis. Tally and record the number of mounted individuals identified and number of slides. "Mounted" organisms are those that were affixed to a slide.

7.12 Record the analytical information at the top of the bench sheet. Record the total number of individuals, number of slides and number of taxa for the sample/replicate.

Note: Slides of freshwater organism parts and mounted individuals should be stored in a slide box. Slides for marine organisms should be kept in a separate slide box.

7.13 For samples that required subsampling, verify the total number of individuals identified is within the target range. If more or fewer than the target number of individuals were identified, refer back to Step 7.4 and 7.5 for resolution. For samples with high proportions of worms, it may be necessary to process additional material because it is not always possible to determine if a worm has a head or should be counted until after it is mounted on a slide.

If the target number of individuals will not be or has not been met with further processing, notify the Taxonomy Supervisor. The Supervisor will notify the client and verify whether the sample was collected under appropriate conditions. Together with the client, a decision will be reached on how to proceed. The outcome is documented as a comment in SBIO and LIMS.

- For a SCI sample, verify that 140-160 organisms were identified per replicate.
- For a LCI or Everglades dipnet sample verify that 100-110 organisms were identified.

7.14 Entry, verification, and authorization of taxonomic data in SBIO and LIMS is performed per SOPs BG-03 and BG-01 by individuals designated by the Taxonomy Supervisor.

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8. DATA ARCHIVAL

8.1 Samples and slides are kept for five years, after which time they may be discarded using appropriate safety procedures. Bench sheets are filed by project and kept indefinitely.

9. QUALITY CONTROL AND DOCUMENTATION

9.1 DEP Laboratory Information Management System (LIMS) and Statewide Biological Database (SBIO2).

9.2 Freshwater Invertebrate, Macroinvertebrate, or Marine Invertebrate bench sheet. Use pen with black ink for all logbook and bench sheet entries.

9.3 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.

9.4 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.

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

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

9.7 Refer to the DEP Laboratory Quality Manual.

10. SAFETY

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

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

10.3 Refer to the DEP Laboratory Health and Safety Plan.

10.4 Refer to the DEP Laboratory Quality Manual.

11. POLLUTION PREVENTION AND WASTE MANAGEMENT

11.1 Refer to the DEP Laboratory Health and Safety Plan.

12. REFERENCES

12.1 DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/lab_sops.htm

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

12.3 DEP SOP FS-7000, *General Biological Community Sampling*

12.4 DEP SOP SCI-1000, *Stream Condition Index Methods*

12.5 DEP SOP BRN-1000, *Biological Reconnaissance Field Methods*

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

12.7 SOP IZ-02, *Stream Condition Index (SCI) Sample Preparation*

12.8 SOP IZ-03, *Invertebrate Hester-Dendy Sample Preparation*

12.9 SOP IZ-04, *Invertebrate Core/Grab/Dredge Sample Preparation*

12.10 SOP IZ-07, *Subsampling of Worms and Midges*

12.11 SOP IZ-08, *Mounting of Worms, Midges, and Aquatic Mites*

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

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

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

12.15 SOP IZ-39, *Macroinvertebrate Subsampling Sample Preparation*

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

12.17 SOP BG-01, *Data-Entry Verification*

12.18 SOP BG-03, *Taxonomic Results and Data Entry*

Appendix of Significant Changes

02/28/2009	Reformatted the layout following SOP LB-001. Expanded section 8. Data Archival
12/31/2009	Added option for wet identification of midges.
12/31/2009	Added website address for list of lowest practical taxonomic level
12/31/2009	Added note to 7.8 to make note on bench sheet of damaged or small individuals for taxa that are usually identified at a lower practical level
12/31/2009	Added definition of “mounted” individual
12/31/2009	Added procedure and specific comments for entering data with too few individuals into LIMS and SBIO.
03/10/2010	Reversed order of Data Archival to SBIO then LIMS.
04/06/2011	Updated website links.
04/02/2014	Updated Sorting SOP #. Formatted for 508 compliance.
04/24/2015	Updated links and SOP references. Moved much of the information previously in Data Archival section into SOP BG-03.
04/26/2016	Removed reference to the Bioassessment Sample Log-in book as it is no longer used for checking sample in and out by taxonomists.
08/10/2017	Standardized SOP name format.
07/25/2019	Clarified language in 7.4.

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07/12/2021 Updated the Health and Safety Plan link in section 12.2.

06/27/2023 Moved “Notes” from 7.6 to 7.2 for better flow of process.

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