Subsampling of Worms and Midges

(Based on Plafkin, et al, 1989, Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-001)

1. SCOPE AND APPLICATION

1.1 This method details the subsampling of worms and midges in freshwater samples collected by quantitative and qualitative means, i.e., Hester-Dendy artificial substrates, Ekman, Van Veen, petite ponar, dipnet or coring devices.

1.2 This method is used for samples collected in freshwater per Standard Operating Procedure (SOP) FS 7000 - *General Biological Community Sampling* and SCI 1000 – *Stream Condition Index Methods*.

1.3 This method is to be used for all samples with Laboratory Information Management System (LIMS) test codes MI-FW-QNAC, -QNNC, -QLDC, -QNCC, -QNCP, or -3COR.

2. SUMMARY OF METHOD

2.1 Worms and midges, each exceeding 100-110 individuals in a single sample or replicate sample, are separated from the other macroinvertebrates in that sample.

2.2 Individuals are randomly selected until a target of 100-110 is reached.

2.3 These individuals will be mounted on slides for identification although some may be identified without mounting if proper Quality Control (QC) proficiency demonstrations are met (see 7.5.1). Proportional counts will be used to calculate the number of each taxon in the sample.

3. APPARATUS AND EQUIPMENT

3.1 Laboratory counter
3.2 Petri dish, 100 x 15 mm, divided radially into 16 sections
3.3 Forceps
3.4 List of random numbers
3.5 Squeeze bottle of 80% Ethanol

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 and SCI 1000.

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

6. SAMPLE PREPARATION PROCEDURE

6.1 Samples are prepared per SOP IZ-02, IZ-03, IZ-04, or IZ-39.

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7. SAMPLE ANALYSIS PROCEDURE

7.1 Samples are analyzed per SOP IZ-06.

7.2 Separate the midges and worms. Count the total number of each group.

7.3 If the total number of organisms in any group exceeds 110, place all members of that group into the sectioned petri dish.

7.4 Use the list of random numbers to pick a section from the Petri dish. Pick each individual out of the chosen section. Once a section has been selected, all organisms within it must be picked. Repeat until the target of 100-110 organisms have been selected. If picking all organisms from a section results in greater than 110 organisms, place the selected organisms in a second sectioned Petri dish. Repeat random selections until the target of 100-110 is reached.

7.4.1 If an individual is straddling the line between 2 sections in the petri dish, it belongs to the section in which its head lies.

7.5 Mount the organisms following SOP IZ-08.

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

- The taxonomist mounting the organisms is the same person who will be identifying the organisms.
- That taxonomist has successfully completed a wet midge identification proficiency demonstration (per SOP IZ-13).

7.6 Record the number of midges or worms subsampled for identification as well as the total number of midges or worms in the sample on the appropriate bench sheet. This step is very important in yielding correct counts. Following identification and enumeration of organisms, the proportional counts will be applied to the total number of midges or worms in the sample.

7.6.1 The subsampling factor is calculated as the # of subsampled midges or worms divided by total # of midges or worms in the sample.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Department of Environmental Protection (DEP) Laboratory Freshwater, Marine Invertebrate, or Macroinvertebrate bench sheet. Use a pen with black ink for all bench sheet entries.

8.1.1 Bench sheets are printed from the DEP Laboratory Information Management System (LIMS) and will usually have been printed during wet identification or during the preparation of the sample.

8.2 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 in the notes section of the benchsheet.

8.3 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create an NCR, open LIMS and go to

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Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to Bureau SOP LB-002.

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

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

8.6 Refer to the Laboratory Quality Manual.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

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

9.3 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.floridadep.net/resources/health-safety-manual

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

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

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

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

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

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

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

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

10.11 SOP -09, *Preparation of 80% Ethanol*

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

10.13 SOP IZ-39, *Non-SCI Macroinvertebrate Sample Preparation*

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

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

10.16 Plafkin, et al, 1989, Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89-001

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Appendix of Significant Changes

12/02/2009	Reformatted the layout following SOP LB-001; added information about the wet midge identification option
11/10/2011	Updated name for the Laboratory Safety Manual
11/04/2013	Updated sorting QC SOP #
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 IZ-LOG form
02/28/2017	Standardized SOP naming conventions
03/14/2018	Updated DEP Laboratory Quality Manual link
02/17/2020	Updated references.
02/08/2021	Updated Health and Safety Plan link

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