Light Trap Ephemeroptera, Plecoptera and Trichoptera (EPT) Sorting, Enumeration and Taxonomic Analysis

1. SCOPE AND APPLICATION

1.1 This method details the sorting, counting and identification of terrestrial adult 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 code MI-FW-QNLT.

2. SUMMARY OF METHOD

Sorting of adult Ephemeroptera, Plecoptera, and Trichoptera (EPT) individuals from light trap samples is conducted using a white developing tray. Sample container is emptied into the developing tray and the adult EPT are placed into labeled vials. The adults are identified to the lowest practical taxonomic level and counted. Taxa and counts are recorded on a bench sheet. Data are 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)

4. REAGENTS AND CHEMICALS

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

Note: Protective clothing, latex or vinyl gloves, and eye protection should be used when working with ethanol.

5. SAMPLE PREPARATION PROCEDURE

5.1 Carefully pour the contents of the ½ gallon sampling jug into a developing tray. Use a squeeze-bottle of ethanol to rinse any remaining insects into the tray.

5.2 Pick out the adult EPTs and place them into a labeled vial.

5.3 Return the residual sample to the ½ gallon sampling jug for further processing if need.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Samples are collected, preserved, and delivered to the lab according to SOP IZ-43 (Light Trap Set-up and Retrieval).

6.2 Samples are checked into the laboratory per SOP IZ-41.

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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 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.

7.3 Until one becomes efficient at working with adult insects, prior to identification, the analyst should 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.

7.4 Wet identification of most EPT taxa can be done using a dissecting microscope. Here are a few useful methods for working with adult insects.

1) If necessary, the abdomen and/or small body parts can be removed and observed with a compound microscope in glycerin using a concavity slide.

2) If necessary, the abdomen can be cleared of tissue by boiling it in 1N potassium hydroxide for 10 minutes and then observed in glycerin.

3) If necessary, the abdomen can be mounted in CMC-1019.

4) If necessary, when trying to determine wing veins, the wing can be removed, spread out in a drop of alcohol on a microscope slide, covered with a cover glass and allowed to dry. Once dry, the veins can be observed using the compound or dissecting microscope.

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.

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.

7.5 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.

7.6 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.

7.7 For slide mounted organisms, write the taxon name directly on the slide label. 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.

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7.8 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.

7.9 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.

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 (SBIO).

9.2 Freshwater Macroinvertebrate 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 AND WASTE MANAGEMENT

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.

10.5 Protective clothing, latex or vinyl gloves, and eye protection should be used when working with ethanol.

13. REFERENCES

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

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

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

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

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

13.6 SOP IZ-41, *Light Trap Sample Arrival/Log-in Procedures*

13.7 SOP IZ-43, *Light Trap Deployment and Retrieval*

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

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

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

Appendix of Changes

12/13/2022	SOP Created
07/25/2024	Added SOP IZ-43 and LB-002 to list of references.
07/07/2025	Added Safety equipment to sections 4 and 10.

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