Standard Operating Procedures
Laboratory Analysis: NEON Benthic Macroinvertebrates

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Table of Revisions

Revision Number	Revision Date	Revisor	Reason for Revision
1	20191127	M. Payne	Including comprehensive language for the NEON project
2	20201111	M. Payne	Revised language regarding QC, stating PTD and PDE will happen prior to data workup

TABLE OF CONTENTS

LIST OF EQUATIONS

This SOP addresses the laboratory operations and analyses for benthic macroinvertebrate samples. This plan describes data quality objectives, measurement and data acquisition, and information management for processing benthic macroinvertebrate samples.

QUALITY OBJECTIVES AND CRITERIA

Sorting Efficacy – Aliquot Method

At least 20% of each sample is re-sorted by a quality control technician, who did not originally sort the sample, to ensure at least 90% of the organisms have been removed. The QCs are performed by technicians who have shown to achieve 90% efficacy on a minimum of 90% of samples they process. QC technicians are trained in the QC process by the sorting lab manager. The QC technician QCs a minimum of 20% of the sorted material from a given sample to ensure at least 90% of the organisms have been removed. The estimated percent efficacy is calculated, using the following equation:

Equation 1. Sorting Efficacy

$$\text{Sorting Efficacy \%} = \left(\frac{Original\ Count}{Origianl\ count + \left(\left(\frac{QC\ count}{QC'd\ Grids} \right) * QC\ total\ girds \right)} \right) * 100$$

Where:
OriginalCount = the number of organisms picked by the first sorter
QCCount = the number of organisms found in the Quality Control sort
QC'd grids = the number of grids sorted during the QC process
QC Total grids = the total number of grids in the QC Caton

Sorting efficacy is measured as the estimated percent of the total organisms found during the original sorting process. If the estimated percent sorting efficacy is 90% or greater, the sample passes the quality control check. If the estimate is less than 90%, the sample is re-sorted. When this happens, the sample undergoes the quality control process again until it passes the 90% efficacy requirement.

Taxonomic Precision and Accuracy

Taxonomic precision is quantified by comparing whole-sample identifications completed by a second taxonomist who did not perform the primary identification. Accuracy of taxonomy is qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species) and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). To calculate taxonomic precision for benthic macroinvertebrate samples, 10% of the samples are randomly selected for re-identification. Comparison of the results of whole sample re-identifications provides a Percent Taxonomic Disagreement (PTD) calculated as:

Equation 2. Percent Taxonomic Disagreement (PTD)

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N} \right) \right] \times 100$$

where
$comp_{pos}$ = the number of agreements
N = the total number of individuals in the larger of the two counts.

The lower the PTD, the more similar taxonomic results are and the overall taxonomic precision is better. A Measurement Quality Objective (MQO) of ≤15% is will be followed for taxonomic differences. Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, the reasons for disagreement investigated, and corrective measures taken where needed.

Sample enumeration is another component of taxonomic precision. Final specimen counts for samples are dependent on the taxonomist, not the rough counts obtained during the sorting activity. Comparison of counts is quantified by calculation of percent difference in enumeration (PDE), calculated as:

Equation 3. Percent Difference in Enumeration

$$PDE = \left(\frac{|Tax1 - Tax2|}{Tax1 + Tax2} \right) \times 100$$

An MQO of ≤5% will be followed. Individual samples exceeding 5% are examined to determine reasons for the exceedance.

MQO Evaluation

PTD and PDE will be calculated by the Lead Freshwater Taxonomists prior to the data for each sample group being validated and handed over for data work up. For samples exceeding these MQOs, corrective actions can include defining the taxa for which re-identification may be necessary (potentially even by a third party), for which samples (even outside of the 10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems.

Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. Where necessary, the Integrated Taxonomic Information System (ITIS, http://www.itis.usda.gov/) will be used to verify nomenclatural validity and spelling. A reference collection will be compiled as the samples are identified.

SAMPLE HANDLING AND CUSTODY

Immediately upon receipt of benthic macroinvertebrate samples, all containers are inspected for damage or leakage. Sample labels are checked against chain of custody forms and/or packing slips and any discrepancies are noted. Receipt records are reported to the client within one business day of sample receipt. Chain of custody logs are reported, throughout the project, according to timelines and methods requested by the client. Samples are logged into the EcoAnalysts, Inc. custom LIMS database and assigned a unique sample tracking number.

Reference collections will be stored until the client provides a location for long term storage. Sample material will be stored in 95% ethanol/5% glycerol at room temperature for six months prior to data delivery for that sample lot. After the six months hold EcoAnalysts will request action for these materials, if no direction is given, use of non-climate control storage may be utilized.

ANALYTICAL METHODS

Sorting Benthic Macroinvertebrate Samples

A sample is checked out by a sorting technician via the LIMS. A sorting bench sheet is printed that contains the EcoAnalysts sample identification information and sorting protocols assigned to it. The sorter records the primary matrix type and approximates the volume of detritus prior to sieving. The standard descriptors for the types of sample matrix are: Inorganic, Coarse Organic, Fine Organic, Vegetation, and Filamentous Algae.

The sample is prepped for subsampling by rinsing the matrix into a 250um mesh sieve. If the sample matrix is made up of a significant percentage of inorganic material, the organic material will be elutriated from the inorganic material prior to sorting.

For elutriation, the whole sample is washed into a shallow pan of water. At this time any large pieces of organic material can be rinsed and inspected thoroughly by the original technician and a secondary technician for attached

and burrowing aquatic invertebrates. If large organic matter is deemed removable from the sample, it is retained separately as sample residues. The sample is agitated with water to separate any organic matter from inorganic sediments. After agitating the sample in water, the lighter organic material is poured back into the sieve. The inorganic portion of the sample remaining in the pan is repeatedly washed and decanted into the sieve until no more organic matter remains in the pan with the inorganic material.

The remaining inorganic sediments are inspected under a magnifying lamp (3X) to look for any invertebrates too heavy to have been elutriated (e.g. mollusks, snails, stone-cased Trichoptera, etc.). If there are significant numbers of heavy invertebrates in the inorganic material – too many to easily remove under the magnifying lamp – the inorganic and organic matrix is recombined into the sieve and entire sample matrix will be prepared for subsample. If there are not significant numbers of heavy invertebrates in the inorganic material, they are removed under the magnifying lamp and placed with the organic matrix. A second technician inspects the inorganic material for organisms until it is determined there are no more invertebrates in the inorganic fraction of the sample. Unless otherwise requested, the inorganic elutriate is discarded.

The organic material and other contents of the sieve are then evenly distributed into the bottom of a Caton-style tray. These are trays of various sizes consisting of uniform grids, each grid being 2 inches per side and the bottom is constructed of 250-micron mesh. A grid (or a standardized portion of a grid) is randomly selected and its contents transferred to a Petri dish. The material in the Petri dish is sorted under a dissecting microscope (minimum magnification = 10X). The benthic macroinvertebrates are counted as they are placed into vials containing 70% ethanol.

Sorters are trained to pick and count only benthic macroinvertebrates, with heads, that were alive during sampling and contain the attributes required for taxonomic identification. Organisms picked may include sub-aquatic organisms or other specified organisms according to the specific study design. Specimens rejected according to EcoAnalysts' standard includes: terrestrials, vertebrates, copepoda, zooplankton, exuviae, and any organism without a head. When the target count of organisms has been reached or the target percentage of the sample has been sorted but not fully sorted, a special large and rare protocol may be followed on any remaining unsorted material. Organisms deemed relatively large or rare to the sample (in comparison with the target taxa enumerated in the final count) are found by a naked eye scan in the unsorted sample remnants and are not counted but picked and placed in a separate vial.

Laser-printed labels containing the appropriate sample tracking information are placed in the vial(s). The total number of organisms removed (not including large and rare organisms), the number of grids sorted out of the total, the time spent sorting, and the final volume of the remaining sample volume are all recorded on the sorting bench sheet, as well as comments significant to the preparation, sorting, and/or condition of the sample.

To ensure every sample meets a standard minimum level of sorting efficacy, standard sorting quality assurance is maintained by re-sorting a portion of the sorted material of every sample that is processed in the lab, and ensuring a minimum efficacy is reached. If a technician is continually not meeting the efficacy requirements of the project, they will be removed from the project. Supplemental project specific guidance that may be provided by the lab manager, such as photo reference guides for rejects.

Taxonomic Identification of Benthic Macroinvertebrates

A taxonomist selects a sample for identification via the LIMS and empties it into a Petri dish. Under a dissecting and/or compound microscope, the invertebrates are identified to the lowest practical level, generally genus/species. The taxonomist enters each taxon directly into the project database using a unique taxonomic code (this is done while at the microscope). The number of individuals of each taxon is counted and entered into the database. As the sample is being identified, the taxonomist enters data directly into the LIMS database and user interface.

The taxonomist measures size class to the nearest whole mm. Size class is measured from the tip of the head to the end of the abdomen. Size class may be extrapolated if the individual is damaged, or may be recorded as “Sample condition” = “Damaged, affecting measurement”.

A synoptic reference collection will be prepared, where at least one specimen (preferably 3-5 specimens) of each taxon encountered is placed into a 1-dram vial containing 70% ethanol and is properly labeled with identity and sample number. Chironomidae reference specimens are permanently slide mounted and labeled with the sample number and taxonomic determination.

10% of the samples are randomly selected for re-identification by a QC taxonomist. All specimens in those samples that were not set aside for the reference collection are re-identified. The final data is adjusted according to the recommendations of both taxonomists. Taxonomic references used for the taxonomic analysis of samples will be provided.

DATA MANAGEMENT

Data is directly entered into the LIMS database. Throughout the project and sample analysis, data entry is double checked for accuracy, and validated by the laboratory managers. The appropriate data are combined for each sample to obtain the sorting statistics and comprehensive taxa lists and counts.

Quality assurance data sheet checks are part of the sample validation process, and include scanning for apparent entry errors, measurement errors, omissions, and anomalies. Suspect data are flagged and/or excluded from use. Data may be presented in table, graph, and chart format. Unusual data are rechecked to verify their accuracy.

Data is formatted on client data ingest sheets and returned by uploading directly to the client project data portal or returning to a filesharing folder.