Standard Operating Procedures
Laboratory Analysis: NEON Zooplankton

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LIST OF EQUATIONS

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

QUALITY OBJECTIVES AND CRITERIA

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 zooplankton 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 1. 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 2. 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

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. A reference collection will be compiled as the samples are identified.

SAMPLE HANDLING AND CUSTODY

Immediately upon receipt of zooplankton 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 40% ethanol at 4°C 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

Preparing Zooplankton Samples

Samples are checked out via LIMS. A sheet is printed out containing all of the sample information and sorting protocols designated for it. Samples are rinsed with 70% ethanol into a graduated container, and the original sample volume is recorded.

Taxonomic Identification and Enumeration of Zooplanktons

After recording the original sample volume, a 1 mL Hensen-Stempel pipette is inserted into the sample and is used to homogenize the sample, mixing it in a random fashion (not swirling). The sub-sample is captured during the mixing process to avoid bias due to sinking of heavier planktonic organisms.

The subsample taken from the homogenized sample is rinsed into a watch glass with 70% ethanol. Based on the organism density of the first 1 mL of the subsample, more 1-mL aliquots are added until the target count of at least 300 zooplankters is present in the watch glass. If the target count is exceeded in one mL of sample, a secondary dilution is made by transferring aliquots from the sample into a second graduated container and diluting this subsample. After recording the volume of the secondary dilution, aliquots are then taken from this secondary dilution for analysis. Identifications are taken to the lowest practical level (Genus and species for Cladocera, Cyclopoida, Calanoida, Anostraca, and Rotifera, family level for Diptera, Hydracarina, and order level for Harpacticoida). The length measurements of 15 individuals each taxon is taken.

The entire contents of the watch glass are counted to allow proper abundance calculations. After identification, enumeration, and measurements, the final volume of the sample (and the secondary dilution, if used) is recorded to calculate the total volume analyzed.

Morphometrics are recorded for a minimum of the first 15 individuals of each taxon identified in a sample. Measurements include: minimum length, maximum length, mean length, mean width (Rotifers only).

After initial analyses are complete, 10% of the samples will be randomly selected for re-identification. The final data will be adjusted according to the recommendations of both taxonomists. A reference collection will be compiled. 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.