Method was extracted from full ASTM Standard Methods protocol

10500 C. Sample Processing and Analysis

1. Sample Processing

After collecting a benthic sample, pour the slurry gradually into a sieve bucket. Gently wash slurry over screen to prevent damaging or losing specimens. Slurries that clog the screen require removal of screened material. A series of one or two coarser screens (e.g., 1-cm and 0.5-cm mesh) will hold back larger materials (e.g., leaves, sticks, shells, and gravel) while permitting organisms and smaller materials to pass through to the bottom sieve. Carefully check rocks, sticks, shells, and other objects for attached or burrowed organisms before discarding. A soft-bristled toothbrush may be used to remove attached invertebrates from rocks, sticks, and similar objects.

Wash residual material on the screen into a container. A cheesecloth bag is useful because it does not restrict the quantity of wash water. Label containers with a collection code but do not affix labels to lids. Similar labels can be written with pencil or indelible ink on high-rag-content paper and placed in the container. Record label code on a field sheet that describes location, date, type of sample, collector's name, and other pertinent information.

Use laboratory elutriation devices,[1,2] as appropriate, to reduce time required to sort benthic organisms from samples containing large amounts of silt, mud, or clay. Wash screened material into a container and fix the contents in a solution of 10% buffered formalin or 70% ethanol.[3–6] If ethanol is used, do not fill more than one-half the container with screened material. Preserve and store animals with calcareous shells or exoskeletons (mussels, snails, crayfish, and ostracods) in 70% ethanol.[6,7]

Some macroinvertebrates (soft-bodied animals) are identified more easily if they are relaxed to prevent constriction during preservation. Common relaxants include carbonated water (soda water) or carbon dioxide added to water. Other relaxants include aqueous solutions of 70% ethyl alcohol, 2% nicotine sulfate, propylene phenoxetol, or 5 to 10% solutions of either chlorotone, chloral hydrate, or magnesium sulfate added gradually to water containing the soft-bodied animals until the degree of relaxation sought is reached. Narcotize organisms before fixing them. Ideally, fix annelid specimens (oligochaetes) in 5 to 10% buffered formalin before preserving them in 70 to 80% ethanol (NOTE: Alcohol is not a satisfactory tissue fixative). Fixation stabilizes tissue proteins to retain characteristics of the soft body (e.g., segmented worms) form.[8,9]

For qualitative samples, place rocks, sticks, and other objects in a white pan partially filled with water. Many animals will float free from these objects and can be removed with forceps.

Assign identification numbers either in the field or at the laboratory and transcribe information from the labels to a permanent ledger. The ledger provides a convenient reference in identifying the number of samples collected at various places, time of sampling, and water characteristics.

Preserve and store in 70% ethanol organisms taken in the field or from artificial substrates and sieved with a U.S. Standard No. 30 sieve. For special studies and to retain anatomical form and structures, fix soft-bodied organisms first with 5 to 10% buffered formalin. **(CAUTION: For health and safety reasons, always take care when using 5 to 10% buffered formalin, or avoid using it to fix or preserve organisms in the field or in the laboratory. Never discard fixatives or preservatives into the environment.)**

2. Sorting and Identification

Whether organisms are sorted in the field or the laboratory, follow consistent procedures. Before processing a sample, transfer information from the label to a data sheet that provides space for scientific names and number of individuals. Place sample directly in a shallow white tray with water for sorting. To facilitate sorting organisms from detritus, the organisms may be stained with rose bengal (200 mg/L or enough to achieve a light pink color) in the formalin or ethanol preservative for at least 24 h.[10] (NOTE: Excessive staining may prevent specific identification of some specimens.) Examine entire sample and separate organisms unless they occur in very large numbers. If a subsample is sorted, take care that rare forms are not excluded. As organisms are picked from the sample, sort under a scanning lens or stereoscopic microscope, separate them into different taxonomic categories, identify to the lowest taxonomic level to meet data quality objectives, and record on the data sheet. Place animals in separate vials according to category and fill vials with 70% ethanol. Inside vials, place labels containing sample tracking number, date collected, sampling location, and names of organisms.

Identify animals in each vial using stereoscopic and compound microscopes (whichever is needed) and available experience and resources. Identify organisms to species level if possible. Additional sources of information on laboratory techniques are available, as well as identification guides and taxonomic keys of macroinvertebrates (see 10500C.4 and Section 10900).

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