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Chapter A4

METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

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This report supersedes TWRI 5A4, published in 1977, entitled "Methods for collection and analysis of aquatic biological and microbiological samples," edited by P.E. Greeson and others.

Revised 1987
Book 5

LABORATORY ANALYSIS

Numerical assessment (relative or semiquantitative method)

(B-5020-85)

Parameters and Codes:
Invertebrates, benthic, wet weight (g/m^2): 70940
Invertebrates, benthic, dry weight (g/m^2): 70941
Invertebrates, benthic, ash weight (g/m^2): 70942
Invertebrates, benthic, total (organisms/m^2): 70943

This method assumes that the objective is to compare the kinds and relative abundances of taxa in samples from several sites or on different sampling dates. The differences between samples are assumed to be directly proportional to differences between the sites or dates. The artificial-substrate method is recommended when collections must be made by persons inexperienced in biology. The procedures described in the "Distribution and Abundance (Quantitative Method)" section also are applicable to sample collection from homogeneous substrates.

1. Applications

The method is applicable to all water and especially is useful for indicating water-quality trends or differences between sites.

2. Summary of method

Benthic invertebrates are collected using uniform procedures throughout a wide area or collected from small, homogeneous areas at sites that are to be compared. Sampling methods include collecting samples, using a dip net, in a standardized manner or for a definite period of time; collecting samples from individual rocks; and using artificial substrates. Unsorted samples, usually containing varying quantities of sand, gravel, and plant detritus, are preserved onsite. In the laboratory, the benthic invertebrates are sorted from the extraneous material, identified, and counted. Biomass is determined if appropriate to the study objectives. Results are reported as numbers of different kinds of benthic invertebrates (taxa) and relative abundance of each taxon for the total collection or for a particular habitat or artificial substrate. Biomass is reported as wet, dry, ash, or ash-free weight.

3. Interferences

Physical factors, such as stream velocity, depth of water, and large rocks, may interfere with sampling in natural substrates. In these places, artificial substrates may provide adequate samples. However, because all sampling methods are selective, all the collections for a particular study must be done in a uniform way. Most samples contain sediment and plant debris from which the invertebrates must be separated. Losses of artificial-substrate samplers to environmental hazards or vandalism may preclude their use at some sites.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Artificial-substrate float*, consisting of a 0.6-m length of polyvinylchloride (PVC) tubing that has a 5-cm inside diameter (ID) and ends sealed (fig. 25). Two clear Plexiglas stabilizer fins are attached near one end and an eyebolt at the other end. One to three multiple-plate samplers are suspended on rods below the float to a depth of 0.3 m measured from the water surface to the midpoint of each sampler.

4.2 *Balance*, capable of weighing to at least 0.1 mg.

4.3 *Barbecue-basket artificial-substrate sampler* (Mason and others, 1967), a cylindrical, welded-wire basket, about 18 cm in diameter and 28 cm long. The basket is filled with 30 rocks, 5 to 8 cm in diameter, or with porcelain spheres that provide interstices for invertebrate colonization and weight for stability (fig. 27). The basket may be placed on the bottom, or it may be suspended above the bottom from a fixed structure or a surface float. A suitable float is a 19-L metal container filled with polyurethane foam.

4.4 *Brush, soft-bristle*, for scrubbing invertebrates from rocks.

4.5 *Collapsible-basket artificial-substrate sampler* (Bull, 1968), consisting of a commercially manufactured basket of coiled wire, bolted to a metal or plastic rim made from 38×3.3 mm stock (fig. 28). The basket is filled with gravel or rock and is covered by a bag of 210 ± 2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives. The basket collapses when lowered onto the streambed but assumes its original shape when raised. The surrounding net prevents escape of invertebrates.

4.6 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.7 *Dip or hand nets* are made in various shapes and sizes,

are sturdy in design, and have a flat side for pressing the net closely against the streambed. Commercial nets are available in various materials and mesh sizes. The desired material and mesh opening should be specified when ordering. Dip nets for general use in the U.S. Geological Survey should have bags of 210±2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives.

4.8 *Drying oven*, thermostatically controlled for use at 105 °C.

4.9 *Forceps*, that have fine or rounded points. Forceps that have fine points are useful for handling small invertebrates. Forceps that have rounded points are less likely to tear netting or puncture the mesh of sieves or other sampling equipment. Forceps are less likely to be lost onsite if marked with bright paint or colored tape.

4.10 *Gloves, waterproof*, Trapper's, shoulder length.

4.11 *Ink, waterproof.*

4.12 *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.13 *Lium sampler*, for individual rocks (Lium, 1974; fig. 23). The sampler consists of a 16-gauge sheet metal hood and an attached conical screen of 210-μm stainless-steel mesh. The base of the hood is padded with flexible foam rubber encased in nylon. The overall dimensions of the sampler are 65 cm long and 45 cm high, including the handle and a base area of 929 cm^2.

4.14 *Microscope*, stereoscopic variable power, 7× to 30×, and *microscope illuminator*. A compound microscope of at least 200× magnification also is useful for taxonomic work.

4.15 *Muffle furnace*, for use at 500 °C.

4.16 *Multiple-plate artificial-substrate sampler*, jumbo modification (Fullner, 1971). The sampler consists of fourteen 7.6-cm square or circular plates of 3.3-mm thick tempered hardboard separated by one or more 2.54-cm square or circular spacers of the same material (fig. 24). Plates 1 to 9 are separated by a single hardboard spacer, plates 9 and 10 are separated by two spacers, plates 10 to 12 are separated by three spacers, and plates 12 to 14 are separated by four spacers. The plates and spacers are held together by a 6.4-mm diameter by 20-cm eyebolt that passes through a hole drilled in the center of each piece.

4.17 *Porcelain crucibles.*

4.18 *Retrieval net*, for multiple-plate sampler (fig. 26). It is a rectangular bag made from a 38-cm square of 210±2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives. The screen-cloth square is folded in half and stitched along two sides. A nylon drawstring serves to secure the top of the net around the eyebolt of the sampler.

4.19 *Sample containers*, plastic or glass, and plastic lids, for transporting unsorted samples to the laboratory. Wide-mouth jars of 120-, 240-, and 475-mL capacity are useful sizes. Sealable plastic bags also may be used for temporary storage of benthic-invertebrate samples.

4.20 *Sieves, U.S. Standard*, 20-cm diameter, and mesh size appropriate to the study objectives. The No. 70 sieve (210-μm mesh opening) has been selected for retaining benthic invertebrates collected as part of the water-quality programs of the U.S. Geological Survey. Sieves that have smaller or larger mesh may be more suitable for some studies. The No. 18 sieve (1,000-μm mesh opening) is useful for removing large rocks and sticks from samples. Stainless-steel mesh is recommended for all sieves because of its greater durability compared to brass.

4.21 *Tub or bucket*, for washing samples or sampling equipment onsite.

4.22 *Vials*, that have plastic poly seal screw lids. Convenient sizes are 7.5-, 15-, and 22-mL capacity.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water.*

5.2 *Glycerin.*

5.3 *Preservative solutions*. Invertebrate samples may be preserved in 70-percent ethyl alcohol, 70-percent isopropyl alcohol, or 4-percent formaldehyde. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.3.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.3.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.3.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

5.3.4 *Formaldehyde.* Dilute 10 mL 37- to 40-percent aqueous formaldehyde solution (formalin) to 100 mL using distilled water.

6. Analysis

6.1 Identify and count the benthic invertebrates in the sample according to taxonomic categories. The degree of identification required (species level is desirable) varies depending on the objectives of the study. A stereoscopic microscope is required; and, for some groups, dissections or microscopic mounts are needed to observe key characteristics. Appropriate reference books (Part 3, "Selected Taxonomic References" section of this report) should be available. The different categories of invertebrates can be placed in separate vials of 70-percent ethyl or 70-percent isopropyl alcohol and can be labeled with the name of the invertebrate and the identification number, date, and origin of the sample. Add a few drops of glycerin or use the ethyl alcohol-glycerin preservative, and seal vial caps if the specimens are to be stored.

6.2 The biomass of benthic invertebrates, expressed as wet, dry, ash, or ash-free weight, is best determined from

samples that were frozen immediately after collection. Biomass determined from alcohol-preserved samples is much less satisfactory (Howmiller, 1972; Stanford, 1973; Donald and Patterson, 1977).

Although generally determined from a total sample, biomass may be determined for an individual taxon. Cases or houses, such as caddisfly larval cases, must be removed from the sample, but shells of mollusks and crustaceans can remain in the sample. If shelled animals constitute 50 percent of the total weight, their weights may be reported separately if only wet weight is required. Separation of the shelled animals is not necessary if wet, dry, and ash weights are to be determined because the ash weight will include the weight of the shells.

6.3 To determine wet weight, remove external water from the invertebrates by blotting for 1 minute on filter paper. Subdivide clumps of invertebrates, but do not separate individuals during blotting. Weigh to 0.1 mg. An alternative method for removing excess liquid is the centrifuge method described by Stanford (1973).

6.4 To determine dry weight, place the invertebrates in a tared porcelain crucible, and dry in an oven at 105 °C to constant weight. Cool in a desiccator and weigh to 0.1 mg. Lower drying temperatures (60 °C) sometimes are used when there is danger of erroneously small values resulting from volatilization or decomposition of fats (Edmondson and Winberg, 1971).

6.5 To determine ash weight, heat the crucible and sample at 500 °C in a muffle furnace to constant weight. Allow at least 1 hour, but some samples will require longer times. Cool and rewet the ash using distilled water to restore the water of hydration of clays and other minerals that may have been lost. Dry at 105 °C to a constant weight. Cool in a desiccator and weigh to 0.1 mg.

7. Calculations

7.1 When only part of the total sample is sorted or counted, project the results from the subsample to the number of specimens in the total sample:

$$\begin{pmatrix}\text{Total number of benthic invertebrates of a particular taxon in sample}\end{pmatrix} = \frac{\text{Number of benthic invertebrates of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of benthic invertebrates of a particular taxon}}{\text{Total number of individuals of all taxa}} \times 100.$$

7.3 Wet weight of benthic invertebrates (grams per sample)

$$= \frac{\text{Wet weight of benthic invertebrates in all samples + Weight of crucible (grams) − Tare weight of crucible (grams)}}{\text{Number of samples}}.$$

7.4 Dry weight of benthic invertebrates (grams per sample)

$$= \frac{\text{Dry weight of benthic invertebrates in all samples + Weight of crucible (grams) − Tare weight of crucible (grams)}}{\text{Number of samples}}.$$

7.5 Ash weight of benthic invertebrates (grams per sample)

$$= \frac{\text{Ash weight of benthic invertebrates in all samples + Weight of crucible (grams) − Tare weight of crucible (grams)}}{\text{Number of samples}}.$$

7.6 Ash-free weight (loss on ignition) of benthic invertebrates (grams per sample)

= Dry weight (grams per sample) − Ash weight (grams per sample).

7.7 Results of sampling from individual rocks are expressed as benthic invertebrates per projected area (aspect) of rock or per total rock surface:

$$\begin{pmatrix}\text{Benthic invertebrates per square meter of projected rock surface}\end{pmatrix} = \frac{\text{Number of benthic invertebrates collected from rock}}{\text{Length of longest axis of rock (millimeters)} \times \text{Length of intermediate axis of rock (millimeters)}} \times 10^6;$$

$$\begin{pmatrix}\text{Benthic invertebrates per square centimeter of total rock surface}\end{pmatrix} = \frac{\text{Number of benthic invertebrates collected from rock}}{\pi\,[\text{length of intermediate axis of rock (millimeters)}]^2} \times 100.$$

8. Reporting of results

8.1 Report the number of taxa present, the percentage composition of each taxon in the sample, and the type of sampling method(s) used. Report biomass to two significant figures.

8.2 Report results in terms of the total sample collected at each sampling site, in a particular habitat, or from the artificial-substrate sampler(s).

9. Precision

No numerical precision data are available.

10. Sources of information

Bull, C.J., 1968, A bottom fauna sampler for use in stony streams: Progressive Fish Culturist, v. 30, p. 119-120.

Donald, G.L., and Patterson, C.G., 1977, Effect of preservation on wet weight biomass of Chironomidae larvae: Hydrobiologia, v. 53, no. 1, p. 75-80.

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Distribution and abundance (quantitative method)

(B-5040-85)

Parameters and Codes:
Invertebrates, benthic, wet weight (g/m^2): 70940
Invertebrates, benthic, dry weight (g/m^2): 70941
Invertebrates, benthic, ash weight (g/m^2): 70942
Invertebrates, benthic, total (organisms/m^2): 70943

1. Applications

This method is used in studies of biological productivity of benthic-invertebrate populations or communities. It is applicable to all natural water.

2. Summary of method

Benthic invertebrates are collected from a defined area using a suitable procedure for removing samples of a known size. A sufficient number of samples is desired to ensure that most of the taxa present are included. Unsorted samples, usually containing varying quantities of sand, gravel, and plant detritus, are preserved onsite. In the laboratory, the benthic invertebrates are separated from the extraneous material, identified, and counted or weighed. Results are reported as numbers of different kinds of benthic invertebrates (taxa) and numbers of individuals in each taxon per unit area of bottom. Biomass is reported as wet, dry, ash, or ash-free weight per unit area of bottom.

3. Interferences

Physical factors, such as stream velocity, depth of water, and large rocks, may interfere with sampling. Most samples contain relatively large quantities of sediment and plant debris from which the invertebrates must be separated. The principal interference with quantitative sampling, however, is the heterogeneity of aquatic habitats and the temporal and spatial variability of the benthic-invertebrate populations (Hynes, 1970).

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, capable of weighing to at least 0.1 mg.

4.2 *Box, drum, or stream-bottom fauna sampler* (Edmondson and Winberg, 1971, p. 69). This is a strong, metal cylinder open at the top and bottom that can be pushed into the sediment to isolate a definite area. The bottom of the cylinder may have a compressible edge to seal against the irregularities of the bed, or the edge may have triangular teeth about 4 cm long, which cut into the bed as the sampler is rotated. Cylindrical samplers can be lengths of stovepipe or 30-cm-diameter aluminum irrigation pipe (Weber, 1973), or they can be constructed to enclose any convenient area as defined by the study objectives and the size of the bed materials. A sample area of 900 to 1,000 cm^2 is common. The maximum practical height for the box is about 75 cm because the collector must be able to reach the bottom with the hands. One of various modifications of the solid cylinder is shown in figure 29. Other modifications are described by Welch (1948), Gerking (1957), Macan (1958), and Waters and Knapp (1961). Depending on the degree of resistance offered to water flow, these devices decrease the tendency for the sampler to cause scour as it approaches the bottom of a stream. Netting should be 210 ± 2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives.

4.3 *Brush, soft-bristle*, small *dip net* of appropriate mesh opening, and a *garden trowel* or small *digging fork* are needed for removing the invertebrates from the substrate enclosed by several of the samplers.

4.4 *Corer*, K.B.-type (fig. 34), or equivalent. Extra weights are available to increase the depth of penetration, and when so used, a winch may be required. These corers have been designed so water passes through during descent but are closed during ascent to prevent loss of sample. In shallow water, a *hand corer* may be used.

4.5 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.6 *Drying oven*, thermostatically controlled for use at 105 °C.

4.7 *Ekman grab*, preferably the tall design (fig. 31), 15×15 cm square, 23 to 30 cm tall. Extra weights are available to increase the depth of penetration. In deep water, the grab is tripped using a messenger; whereas, in shallow water, the Ekman grab may be operated using a handle.

4.8 *Forceps*, that have fine or rounded points. Forceps that have fine points are useful for handling small invertebrates. Forceps that have rounded points are less likely to tear netting or puncture the mesh of sieves or other sampling

equipment. Forceps are less likely to be lost onsite if marked with bright paint or colored tape.

4.9 *Gloves, waterproof,* Trapper's, shoulder length.

4.10 *Ink, waterproof.*

4.11 *Labels, waterproof,* or labels may be cut from sheets of plastic paper.

4.12 *Microscope,* stereoscopic variable power, 7× to 30×, and *microscope illuminator.* A compound microscope of at least 200× magnification also is useful for taxonomic work.

4.13 *Muffle furnace,* for use at 500 °C.

4.14 *Ponar grab* (fig. 32), or *screen-top sediment sampler.* These grabs trip on contact with the bottom and have been designed so water passes through to lessen the shock wave (Flannagan, 1970; Hudson, 1970). Word and others (1976) reported improved performance when the fixed panels were replaced by hinged screen panels. Accessory weights may be used, and these grabs should be operated with a winch. When empty, the grab is about 23 kg without weights and about 32 kg with weights.

4.15 *Porcelain crucibles.*

4.16 *Sample containers,* plastic or glass, and plastic lids, for transporting unsorted samples to the laboratory. Wide-mouth jars of 120-, 240-, and 475-mL capacity are useful sizes. Sealable plastic bags also may be used for temporary storage of benthic-invertebrate samples.

4.17 *Sieves, U.S. Standard,* 20-cm diameter, and mesh size appropriate to the study objectives. The No. 70 sieve (210-μm mesh opening) has been selected for retaining benthic invertebrates collected as part of the water-quality programs of the U.S. Geological Survey. Sieves that have smaller or larger mesh may be more suitable for some studies. The No. 18 sieve (1,000-μm mesh opening) is useful for removing large rocks and sticks from samples. Stainless-steel mesh is recommended for all sieves because of its greater durability compared to brass.

4.18 *Surber sampler* (fig. 30). This sampler commonly has been used in stream studies, although the enclosed box-type samplers, such as the *portable invertebrate box sampler* are preferred, if available. Modifications of the Surber sampler (Waters and Knapp, 1961; Withers and Benson, 1962; Mundie, 1971) eliminated many deficiencies of the original design. Netting used in the construction or operation of these samplers should be 210±2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives.

4.19 *Tape, plastic,* or *paraffin* for sealing jar and vial lids.

4.20 *Tub or bucket,* for washing samples or sampling equipment onsite.

4.21 *Van Veen grab* (fig. 33), weighs 48 kg and may be loaded with additional weights. The grab has a capacity of 40 L and samples an area of 1,500 cm^2. Screened panels enable water to flow through during descent to lessen the shock wave on the bottom. Rubber flaps cover the screened openings to prevent sediment washout during recovery.

4.22 *Vials,* that have plastic poly seal screw lids. Convenient sizes are 7.5-, 15-, and 22-mL capacity.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water.*

5.2 *Glycerin.*

5.3 *Preservative solutions.* Invertebrate samples may be preserved in 70-percent ethyl alcohol or 70-percent isopropyl alcohol. Formaldehyde solution is not recommended. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.3.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.3.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.3.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

6. Analysis

6.1 Identify and count the benthic invertebrates in the sample according to taxonomic categories. The degree of identification required (species level is desirable) varies depending on the objectives of the study. A stereoscopic microscope is required; and, for some groups, dissections or microscopic mounts may be needed to observe key characteristics. Appropriate reference books (Part 3, "Selected Taxonomic References" section of this report) should be available. The different categories of invertebrates can be placed in separate vials of 70-percent ethyl or 70-percent isopropyl alcohol and can be labeled with the name of the invertebrate and the identification number, date, and origin of the sample. Add a few drops of glycerin or use the ethyl alcohol-glycerin preservative, and seal vial caps if the specimens are to be stored.

6.2 The biomass of benthic invertebrates, expressed as wet, dry, ash, or ash-free weight, is best determined from samples that were frozen immediately after collection. Biomass determined from alcohol-preserved samples is much less satisfactory (Howmiller, 1972; Stanford, 1973; Donald and Patterson, 1977).

Although generally determined from the total sample, biomass may be determined for an individual taxon. Cases or houses, such as caddisfly larval cases, must be removed from the sample, but shells of mollusks and crustaceans can remain in the sample. If shelled animals constitute 50 percent of the total weight, their weights may be reported separately if only wet weight is required. Separation of the shelled animals is not necessary if wet, dry, and ash weights are to be determined because the ash weight will include the weight of the shells.

6.3 To determine wet weight, remove external water from the invertebrates by blotting for 1 minute on filter paper. Subdivide large clumps of invertebrates, but do not separate individuals during blotting. Weigh to 0.1 mg. An alternative

method for removing excess liquid is the centrifuge method described by Stanford (1973).

6.4 To determine dry weight, place the invertebrates in a tared porcelain crucible, and dry in an oven at 105 °C to a constant weight. Cool in a desiccator and weigh to 0.1 mg. Lower drying temperatures (60 °C) sometimes are used when there is danger of erroneously small values resulting from volatilization or decomposition of fats (Edmondson and Winberg, 1971).

6.5 To determine ash weight, heat the crucible and sample at 500 °C in a muffle furnace to a constant weight. Allow at least 1 hour, but some samples will require longer times. Cool and rewet the ash using distilled water to restore the water of hydration of clays and other minerals that may have been lost. Dry at 105 °C to a constant weight. Cool in a desiccator and weigh to 0.1 mg.

7. Calculations

7.1 When only part of the total sample is sorted or counted project the results from the subsample to the number of specimens in the total sample:

$$\begin{matrix}\text{Total number of benthic invertebrates of a particular taxon in sample}\end{matrix} = \frac{\text{Number of benthic invertebrates of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Number of benthic invertebrates per square meter

$$= \frac{\text{Number of benthic invertebrates in all samples}}{\text{Area of sampler (square meters)} \times \text{Number of samples}}.$$

7.3 Wet weight of benthic invertebrates (grams per square meter)

$$= \frac{\text{Wet weight of benthic invertebrates in all samples} + \text{Weight of crucible (grams)} - \text{Tare weight of crucible (grams)}}{\text{Area of sampler (square meters)} \times \text{Number of samples}}.$$

7.4 Dry weight of benthic invertebrates (grams per square meter)

$$= \frac{\text{Dry weight of benthic invertebrates in all samples} + \text{Weight of crucible (grams)} - \text{Tare weight of crucible (grams)}}{\text{Area of sampler (square meters)} \times \text{Number of samples}}.$$

7.5 Ash weight of benthic invertebrates (grams per square meter)

$$= \frac{\text{Ash weight of benthic invertebrates in all samples} + \text{Weight of crucible (grams)} - \text{Tare weight of crucible (grams)}}{\text{Area of sampler (square meters)} \times \text{Number of samples}}.$$

7.6 Ash-free weight (loss on ignition) of benthic invertebrates (grams per square meter)

= Dry weight (grams per square meter)
− Ash weight (grams per square meter).

8. Reporting of results

8.1 Report as follows: less than 100 benthic invertebrates per square meter, nearest whole number; 100 benthic invertebrates or more, two significant figures. Report biomass to two significant figures.

8.2 Report results in terms of a unit area of the habitat sampled.

9. Precision

No numerical precision data are available.

10. Sources of information

Donald, G.L., and Patterson, C.G., 1977, Effect of preservation on wet weight biomass of Chironomidae larvae: Hydrobiologia, v. 53, no. 1, p. 75-80.

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bottom fauna sampler: Proceedings of the West Virginia Academy of Science, v. 34, p. 16-20.

Word, J.Q., Kauwling, T.J., and Mearns, A.J., 1976, A comparative field study of benthic sampling devices used in southern California benthic surveys—A task report for EPA: Corvallis, Oreg., U.S. Environmental Protection Agency, EPA Grant R 801152, 79 p.

Invertebrate drift

(B-5050-85)

Parameters and Codes: Not available

Because drifting invertebrates come from a variety of habitats, drift samples generally contain a large variety of taxa (Waters, 1961; Larimore, 1974; Slack and others, 1976). Benthic invertebrates respond to stresses of pollution, flood, drought, or insecticides by increased drifting; therefore, drift may be a useful indicator of water quality. Drift is a source of invertebrates for colonization of artificial-substrate samplers and for recolonization of depopulated areas of streams.

1. Applications

The method is applicable to all flowing water in which the velocity is at least 0.01 m/s.

2. Summary of method

Drifting invertebrates carried by flowing water are caught in a stationary net. Because the catch increases as the volume of water passing through the net increases, drift results are expressed as density (number of invertebrates or biomass per unit volume of water), as drift rate (number of invertebrates or biomass passing a sampling point in unit time), or as total daily drift rate (total number of invertebrates or biomass passing a given point in 24 hours).

3. Interferences

Drift nets may become clogged with ice, detritus, tree leaves, or sediment causing backflow and decreased sampling efficiency. If the opening of the net is in contact with the stream bottom, nondrifting invertebrates may be caught; if the opening extends above the surface, many adults and terrestrial invertebrates may be caught. Sufficient current must be present to carry the actively or passively drifting invertebrates into the net. If only naturally occurring drift rates are to be determined, nets should be installed upstream from disturbances caused by human activity, cattle, or other sources of artificially created invertebrate drift. Because drifting activity for many species varies greatly during a diel cycle, comparative collections should be made during similar time periods.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Balance*, capable of weighing to at least 0.1 mg.

4.2 *Current meter, pygmy,* or *digital flowmeter.*

4.3 *Desiccator*, containing silica gel or anhydrous calcium sulfate.

4.4 *Drift net* (fig. 35), 30×30 cm, 15×30 cm, or 30×46 cm, that has anchor rods and clamps. Bag nets, 1 m or more in length, should have 210±2-μm mesh-opening nylon or polyester monofilament screen cloth, unless otherwise indicated by the study objectives. The percent open area of the netting should be as large as possible to facilitate flowthrough and decrease backflow. A net that is cylindrical for most of its length is less liable to clog than one that is tapered (Waters, 1969).

4.5 *Drying oven*, thermostatically controlled for use at 105 °C.

4.6 *Forceps*, that have fine or rounded points. Forceps that have fine points are useful for handling small invertebrates. Forceps that have rounded points are less likely to tear netting or puncture the mesh of sieves or other sampling equipment. Forceps are less likely to be lost onsite if marked with bright paint or colored tape.

4.7 *Ink, waterproof.*

4.8 *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.9 *Microscope*, stereoscopic variable power, 7× to 30×, and *microscope illuminator.* A compound microscope of at least 200× magnification also is useful for taxonomic work.

4.10 *Muffle furnace*, for use at 500 °C.

4.11 *Porcelain crucibles.*

4.12 *Sample containers*, plastic or glass, and plastic lids, for transporting unsorted collections to the laboratory. Wide-mouth jars of 120-, 240-, and 475-mL capacity are useful sizes. Sealable plastic bags also may be used for temporary storage of benthic-invertebrate samples.

4.13 *Sieves, U.S. Standard*, 20-cm diameter, and mesh size appropriate to the study objectives. The No. 70 sieve (210-μm mesh opening) has been selected for retaining benthic invertebrates collected as part of the water-quality programs of the U.S. Geological Survey. Sieves that have smaller or larger mesh may be more suitable for some studies. The No. 18 sieve (1,000-μm mesh opening) is useful for removing large rocks and sticks from samples. Stainless-steel mesh is recommended for all sieves because of its greater durability compared to brass.

4.14 *Tape, plastic*, or *paraffin*, for sealing jar and vial lids.

4.15 *Vials*, that have plastic poly seal screw lids. Convenient sizes are 7.5-, 15-, and 22-mL capacity.

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Distilled or deionized water.*

5.2 *Glycerin.*

5.3 *Preservative solutions.* Drift invertebrate samples may be preserved in 70-percent ethyl alcohol or 70-percent isopropyl alcohol. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.3.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.3.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.3.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

6. Analysis

6.1 Identify and count the benthic invertebrates in the sample according to taxonomic categories. The degree of identification required (species level is desirable) varies depending on the objectives of the study. A stereoscopic microscope is required; and, for some groups, dissections or microscopic mounts are needed to observe key characteristics. Appropriate reference books (Part 3, "Selected Taxonomic References" section of this report) should be available. The different categories of invertebrates can be placed in separate vials of 70-percent ethyl or 70-percent isopropyl alcohol and can be labeled with the name of the invertebrate and the identification number, date, and origin of the sample. Add a few drops of glycerin or use the ethyl alcohol-glycerin preservative, and seal vial caps if the specimens are to be stored.

6.2 The biomass of drift invertebrates, expressed as wet, dry, ash, or ash-free weight, is best determined from samples that were frozen immediately after collection. Biomass determined from alcohol-preserved samples is much less satisfactory (Howmiller, 1972; Stanford, 1973; Donald and Patterson, 1977).

Although generally determined from the total sample, biomass may be determined for an individual taxon. Cases or houses, such as caddisfly larval cases, must be removed from the sample, but shells of mollusks and crustaceans can remain in the sample. If shelled animals constitute 50 percent of the total weight, their weights may be reported separately if only wet weight is required. Separation of the shelled animals is not necessary if wet, dry, and ash weights are to be determined because the ash weight will include the weight of the shells.

6.3 To determine wet weight, remove external water from the animals by blotting for 1 minute on filter paper. Subdivide large clumps of invertebrates, but do not separate individuals during blotting. Weigh to 0.1 mg. An alternative method of removing excess liquid is the centrifuge method described by Stanford (1973).

6.4 To determine dry weight, place the invertebrates in a tared porcelain crucible, and dry in an oven at 105 °C to a constant weight. Cool in a desiccator and weigh to 0.1 mg. Lower drying temperatures (60 °C) sometimes are used when there is danger of erroneously small values resulting from volatilization or decomposition of fats (Edmondson and Winberg, 1971).

6.5 To determine ash weight, heat the crucible and sample at 500 °C in a muffle furnace to a constant weight. Allow at least 1 hour, but some samples will require longer times. Cool and rewet the ash using distilled water to restore the water of hydration of clays and other minerals that may have been lost. Dry at 105 °C to a constant weight. Cool in a desiccator and weigh to 0.1 mg.

7. Calculations

7.1 When only part of the total sample is sorted or counted, project the results from the subsample to the number of specimens in the total collection:

$$\text{Number of drift invertebrates of a particular taxon in sample} = \frac{\text{Number of taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of drift invertebrates of a particular taxon}}{\text{Total number of drift invertebrates of all taxa}}.$$

Weight calculations may be on a sample basis or a daily (24 hour) basis depending on the study objectives.

7.3 Wet weight of drift invertebrates (grams)

= Wet weight of drift invertebrates
+ Crucible (grams)
− Tare weight of crucible (grams).

7.4 Dry weight of drift invertebrates (grams)

= Dry weight of drift invertebrates
+ Crucible (grams)
− Tare weight of crucible (grams).

7.5 Ash weight of drift invertebrates (grams)

= Ash weight of drift invertebrates
+ Crucible (grams)
− Tare weight of crucible (grams).

7.6 Ash-free weight (loss on ignition) of drift invertebrates (grams)

= Dry weight (grams)

− Ash weight (grams).

Invertebrate drift density and rate may be expressed on a sample basis or a daily (24 hour) basis depending on the study objectives (Waters, 1969, 1972; Elliott, 1970).

7.7 Drift density (number or grams per cubic meter)

$$= \frac{\text{Quantity of drift invertebrates (number or grams)}}{\text{Volume of water sampled (cubic meters)}}.$$

7.8 Drift rate (number or grams per time)

$$= \frac{\text{Quantity of drift invertebrates (number or grams)}}{\text{Volume of water sampled (cubic meters)} \times \text{Stream discharge (cubic meters per time)}}.$$

7.9 Total daily drift rate (number or grams per 24 hours)

$$= \frac{\text{Total daily quantity of drift invertebrates (number or grams)}}{\text{Volume of water sampled (cubic meters)} \times \text{Total stream discharge (cubic meters per 24 hours)}}.$$

8. Reporting of results

Report drift quantity, taxa, and methods of collection for daylight samples. If sampling was done for 24 hours, report drift quantity and taxa per unit volume and time to indicate any periodicity that occurred. Describe methods of collection.

9. Precision

No numerical precision data are available.

10. Sources of information

Donald, G.L., and Patterson, C.G., 1977, Effect of preservation on wet weight biomass of Chironomidae larvae: Hydrobiologia, v. 53, no. 1, p. 75-80.

Edmondson, W.T., and Winberg, G.G., eds., 1971, A manual on methods for the assessment of secondary productivity in fresh waters: Oxford and Edinburgh, Blackwell Scientific Publications, International Biological Programme Handbook 17, 358 p.

Elliott, J.M., 1970, Methods of sampling invertebrate drift in running water: Annales de Limnologie, v. 6, p. 133-159.

Howmiller, R.P., 1972, Effects of preservatives on weights of some common macrobenthic invertebrates: Transactions of the American Fisheries Society, v. 101, p. 743-746.

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Slack, K.V., Nauman, J.W., and Tilley, L.J., 1976, Evaluation of three collecting methods for a reconnaissance of stream benthic invertebrates: Journal of Research of the U.S. Geological Survey, v. 4, no. 4, p. 491-495.

Stanford, J.A., 1973, A centrifuge method for determining live weights of aquatic insect larvae, with a note on weight loss in preservative: Ecology, v. 54, p. 449-451.

Waters, T.F., 1961, Standing crop and drift of stream bottom organisms: Ecology, v. 42, p. 532-537.

_____ 1969, Invertebrate drift-ecology and significance to stream fishes, *in* Northcote, T.G., ed., Symposium on salmon and trout in streams: Vancouver, University of British Columbia, p. 121-134.

_____ 1972, The drift of stream insects: Annual Review of Entomology, v. 17, p. 253-272.

Permanent-slide method for larvae of Chironomidae

(B-5200-85)

Parameter and Code: Not applicable

Chironomidae (midges) is a family of the insect Order Diptera (two-winged flies), and the immature stages are principally aquatic. The larvae, which are found in all kinds of water except the open ocean, make up a substantial part of most freshwater-invertebrate communities (Roback, 1957). They are important as a source of fishfood and are considered to be useful indicators of water quality. Chironomids are holometabolous (have complete metamorphosis). The larva, which is the feeding stage or most active phase of the chironomid life cycle, has a complete head capsule that is nonretractable within the thorax, and the mandibles are opposed (fig. 37). It has prolegs (not true insect legs) at both ends of the soft, wormlike body. The anterior prolegs are just behind the head capsule on the ventral side of the first thoracic segment and often are fused for their entire length. The posterior prolegs on the last abdominal segment are never fused. The larvae lack spiracles (respiratory openings in the abdominal walls). In some species, ventral gills, called blood gills, are just anterior to the posterior prolegs.

Some chironomid larvae move freely in water, but the larvae of many species live in tubes that they build from algae, fine sediment, and bits of plant debris bound or cemented together with a salivary secretion (fig. 38). Commonly, these structures have the appearance of sand tubes attached to rocks or other solid objects. Both ends of the tubes are open, and the larvae circulate water through them by undulating their bodies. The larvae feed on diatoms and other algae, organic detritus, microcrustaceans, and other midge larvae.

Adult chironomids are small, delicate, gnatlike, nonbiting flies (10 mm long) that are found in swarms by bodies of water, especially in the evening, and near lights at night. The life cycles of the insects are variable; some forms have only one generation every 2 years, while others have several generations during a year.

Identification of chironomid larvae is based mainly on the mouth parts that can be seen only through a microscope. The method described is a modification of procedures developed by Mason (1968, 1970) and Beck (1976) and is suitable for most chironomid larvae.

Some investigators, especially those who are working with chironomid systematics, dissect their larval specimens. They mount just the head capsules, and sometimes they dissect the head capsule and mount certain mouth parts separate from the head under one cover glass.

1. Applications

The method is suitable for all chironomid larvae.

2. Summary of method

Chironomidae larvae from a benthic-invertebrate sample are sorted into visually distinct groups. Representative specimens are heated in 10-percent potassium hydroxide solution

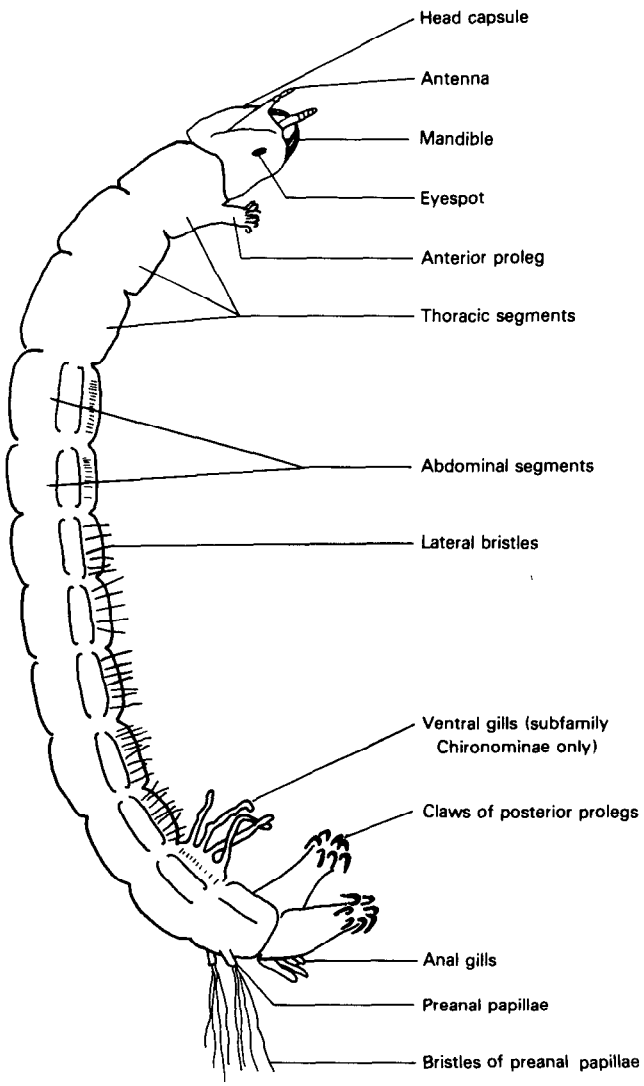
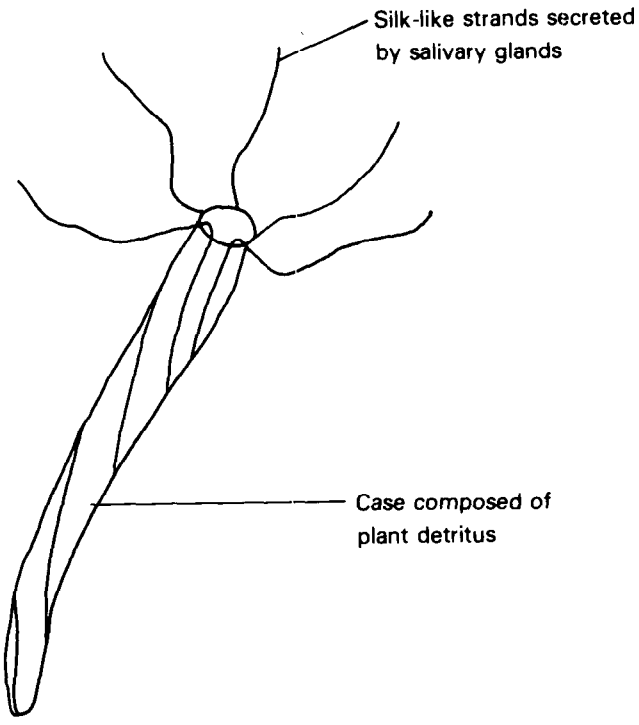
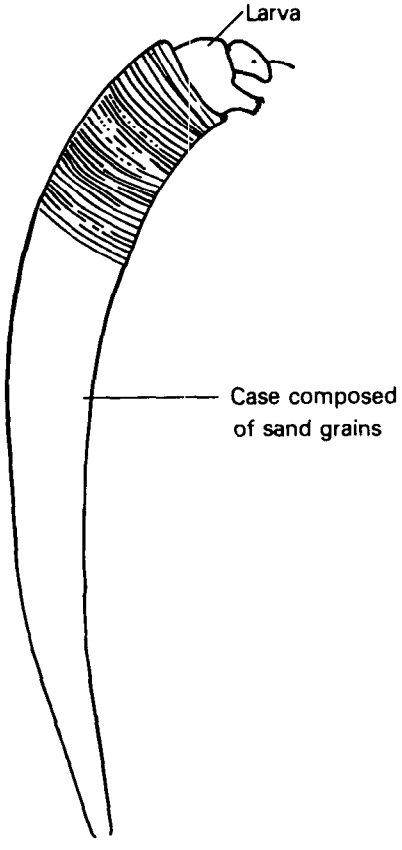


Figure 37.—Idealized external features of a larva of the Family Chironomidae. Features are from more than one subfamily.



Rheotanytarsus pusio



Stempellina spp.

Figure 38.—Examples of cases constructed by larvae of the Family Chironomidae.

to dissolve soft body tissues, placed ventral side up on a microscope slide in a mounting medium, and pressed under a cover glass. The mounted specimens are identified. The number of taxa and individuals in each taxon are tabulated and reported as a percentage of the benthic-invertebrate population or reported in other ways appropriate to the study objectives.

3. Interferences

Heating time is critical. If not heated long enough, the specimen may be too opaque for examination; if heated too long, the specimen will be too transparent and difficult to manipulate during mounting procedures. Sand and other material that cannot be removed by heating may be forced from the gut into the mouth when pressed, obscuring the mouth parts. Too much pressure during mounting may damage diagnostic features shown in figures 39 and 40.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Cover glasses*, circular, No. 1 or 2, 12-mm diameter.

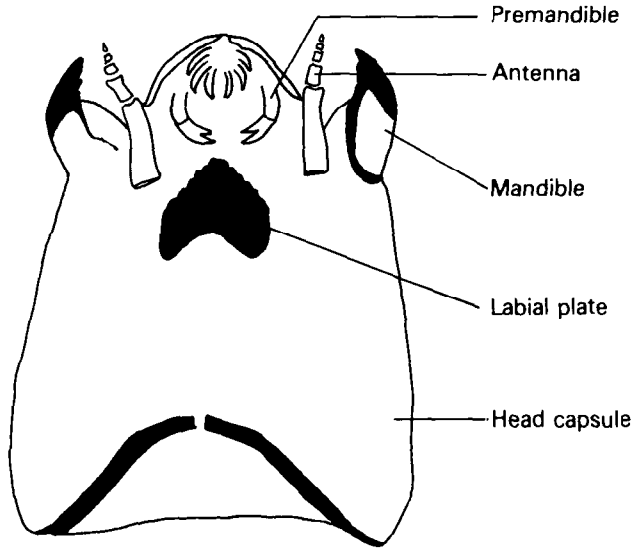
4.2 *Crucibles*, high-form, porcelain, 10-mL capacity.

4.3 *Forceps*, blunt curved tips, and *microforceps*, fine-tipped.

4.4 *Hotplate*, electric.

4.5 *Labels*, for microscope slides. When many slides are prepared, information about the source of the sample can be typed on sheets of paper, photocopied and reduced one-half or two-thirds in size, cut out, and glued onto slides using white glue, or equivalent. *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.6 *Marking pen*, permanent, waterproof, for labeling slides.



Eukiefferiella spp.

Figure 39.—Ventral view of larval head capsule of the Subfamily Orthocladiinae, simplified.

4.7 *Microscope, compound*, preferably having differential interference contrast capable of 1,000× magnification.

4.8 *Microscope slides*, glass, precleaned, 25×75 mm.

4.9 *Needles, pins, and probes*, for manipulating specimens under a stereomicroscope.

4.10 *Ocular micrometer*, graduated to 5 μm.

4.11 *Spot plates*, white porcelain.

4.12 *Stereoscopic zoom microscope* (dissecting), capable of 80× magnification.

4.13 *Vials*, 4 mL, and poly seal screw lids.

4.14 *White glue.*

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Acetic acid*, glacial.

5.2 *Distilled or deionized water.*

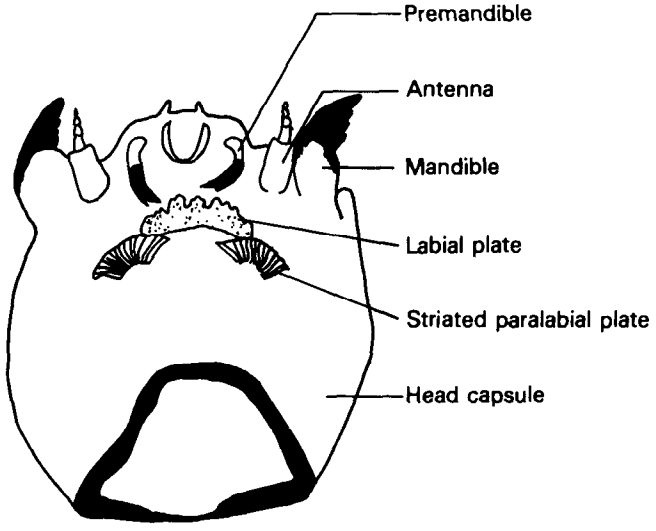
5.3 *Fingernail polish*, clear.

5.4 *Glycerin.*

5.5 *Mounting medium*, CMC-10, or prepare medium as follows: In 50 mL distilled water, dissolve 30 g Gum arabic (amorphic), 200 g chloral hydrate, and 20 mL glycerin. Completely dissolve each solid ingredient before adding succeeding reagents. Filter final mixture through clean cheese cloth.

5.6 *Potassium hydroxide solution*, 10 percent. Dissolve 10 g potassium hydroxide (KOH) pellets in 100 mL distilled water.

5.7 *Preservative solutions.* Samples may be preserved in 70-percent ethyl alcohol or 70-percent isopropyl alcohol. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:



Chironomus spp.

Figure 40.—Ventral view of larval head capsule of the Subfamily Chironominae, simplified. Notice that the left mandible is turned outward; changes in position of structures are common during mounting procedures.

5.7.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.7.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.7.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

6. Analysis

Usually, time does not permit mounting all chironomids in a sample, so the results from a subsample are used to calculate the distribution of taxa and individuals in the original sample. The size of the subsample to be mounted for microscopic examination will depend on the size of the sample, the number of visually distinct groups, and the study objectives.

6.1 Using a stereoscopic microscope, separate the total sample into groups on the basis of general appearance and external features. Some morphological features most useful for separating specimens into groups are:

6.1.1 Body characteristics:

a. Length.
b. Color and color distribution.
c. Enlarged sections.
d. Presence or absence of blood gills.
e. Preanal papillae and bristles.

6.1.2 Head-capsule characteristics:

a. Length and width.
b. Color and darkened areas, such as mouth parts.
c. Number, shape, and arrangements of eyespots.
d. Shape and unusual appendages.

Individual depressions on porcelain spot plates are convenient compartments for separating the subsamples of larvae.

6.2 Randomly select representatives of each group for mounting. For small groups of 10 or fewer individuals, mount a subgroup of 5, or at least 50 percent. For larger groups, remove a subgroup by stratified random sampling and cluster or two-stage sampling. Store the unmounted specimens in vials of 70-percent ethyl alcohol containing one drop of glycerin.

6.3 Place subgroups in depressions of a spot plate filled with distilled water, and soak 10 minutes to remove the alcohol.

6.4 Transfer the subgroups to another spot plate or to crucibles containing 10-percent KOH (Note 1). Heat for 10 to 15 minutes or until the bodies are semitransparent and noticeably lighter in color. (CAUTION.—Excessive heating results in too much digestion of the soft parts, making the specimens too transparent and difficult to see and to manipulate). While heating, add distilled water to the KOH solution to compensate for evaporation.

Note 1: Use fresh KOH solution for each subgroup.

6.5 Transfer the specimens from the KOH solution to a clean spot plate of distilled water (Note 2) for at least 3 minutes to remove the KOH.

Note 2: Residual KOH can make the specimens too soft,

thus interfering with the mounting medium. Instead of the water rinse, glacial acetic acid can be used to neutralize the KOH if residual KOH is a problem.

6.6 Transfer the specimens to another spot plate of 95-percent ethyl alcohol for 3 to 5 minutes. This treatment removes the water or acetic acid and makes the specimen crisp, which results in optimum distribution of mouth parts in the final preparation.

6.7 Place a small drop of mounting medium on a clean glass microscope slide. Position one specimen in the drop of medium, ventral side up, and if necessary, move the specimen using a dissecting needle and microforceps. Place a 12-mm diameter cover glass on the drop containing a specimen and, using a stereoscopic microscope, use the cover glass and the viscous mounting medium to roll, slide, or push each specimen so it lies flat, ventral side up. Apply additional pressure to spread the mouth parts. Allow preparation to dry for 1 week, keeping the slide horizontal (Note 3).

Note 3: With practice, this procedure can be effective for processing many specimens. Chironomids larger than the 12-mm cover glass should be cut in half and mounted under one or two cover glasses.

6.8 Specimens may dry after 2 or 3 years in the mounting medium unless the edges of the cover glass are sealed. To make the preparations more permanent, ring the slide by coating the edges of the cover glass and any exposed mounting medium with clear fingernail polish.

7. Calculations

7.1 When only part of the total sample of Chironomidae larvae is mounted and identified, project the results from those mounted to the total number of specimens:

$$\text{Total number of individuals of a particular taxon in sample} = \frac{\text{Number of individuals of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of individuals of a particular taxon}}{\text{Total number of individuals of all taxa}} \times 100.$$

8. Reporting of results

Report the number of taxa present, the number and percentage of individuals in each taxon in the sample, and the method of collection.

9. Precision

No numerical precision data are available.

10. Sources of information

Beck, W.M., Jr., 1976, Biology of the larval chironomids: State of Florida, Department of Environmental Regulation Technical Series, v. 2, no. 1, 58 p.

Mason, W.T., Jr., 1968, An introduction to the identification of chironomid larvae: U.S. Department of the Interior, Federal Water Pollution Control Administration, Division of Pollution Surveillance, 89 p.

——— 1970, Preparing adult Chironomidae for identification: U.S. Department of the Interior, Federal Water Quality Administration, Analytical Quality Control Laboratory Newsletter 6, p. 10.

Roback, S.S., 1957, The immature tendipedids of the Philadelphia area: Monographs of the Academy of Natural Sciences of Philadelphia 9, 152 p.

Method for identification of immature Simuliidae

(B-5220-85)

Parameter and Code: Not applicable

Larvae and pupae of the insect Family Simuliidae (blackflies) commonly are abundant in swiftly flowing freshwater streams having cobble or gravel bottom. They occur in reaches that have smooth, relatively laminar flow as opposed to reaches that have pools, eddies, or turbulence (Hynes, 1970).

Simuliids are members of the insect Order Diptera (two-winged flies), and as adults can be a serious nuisance to man and animals, especially during the summer months when they emerge and swarm in great numbers. These humpbacked blackflies can inflict a stinging bite that may be followed by intense itching and sometimes bleeding. Severe attacks by blackflies have been known to cause the death of livestock from shock and loss of blood. Blackfly attacks also have been reported to cause a decrease in milk production at dairy farms. Some species of blackflies transmit human onchocerciasis, and other species transmit certain protozoan and other filarial organisms that cause diseases in birds.

Simuliids, like other dipterans, undergo complete metamorphosis (holometabolous). The adults are small and robust, usually dark-colored, and have broad wings, which have large anterior veins. An extensive taxonomic literature about the adults has been stimulated by the economic importance of blackflies. However, until recently, little research was done on the taxonomy of the immature forms.

The immature stages, larvae and pupae, are strictly aquatic. The pupae are enclosed in vaselike or slipperlike cases (fig. 41) attached to rocks, debris, or other solid objects. The pupae have a pair of conspicuous respiratory organs on the thorax and filaments numbering from 2 to 60 (fig. 42). The filaments protrude from the open end of the pupal case. Usually, a pair of prominent terminal hooks is on the last abdominal segment (fig. 42).

The larvae measure 3 to 15 mm in length and are attached to stones or other substrates. The larva is characterized by a soft body that is swollen posteriorly, a pair of mouth fans, one anterior proleg, and a posterior crochet ring composed of minute hooks (fig. 43) by which it adheres to the substrate. The larva moves in a looping manner by means of the posterior crochet ring and anterior proleg. A strand of sticky

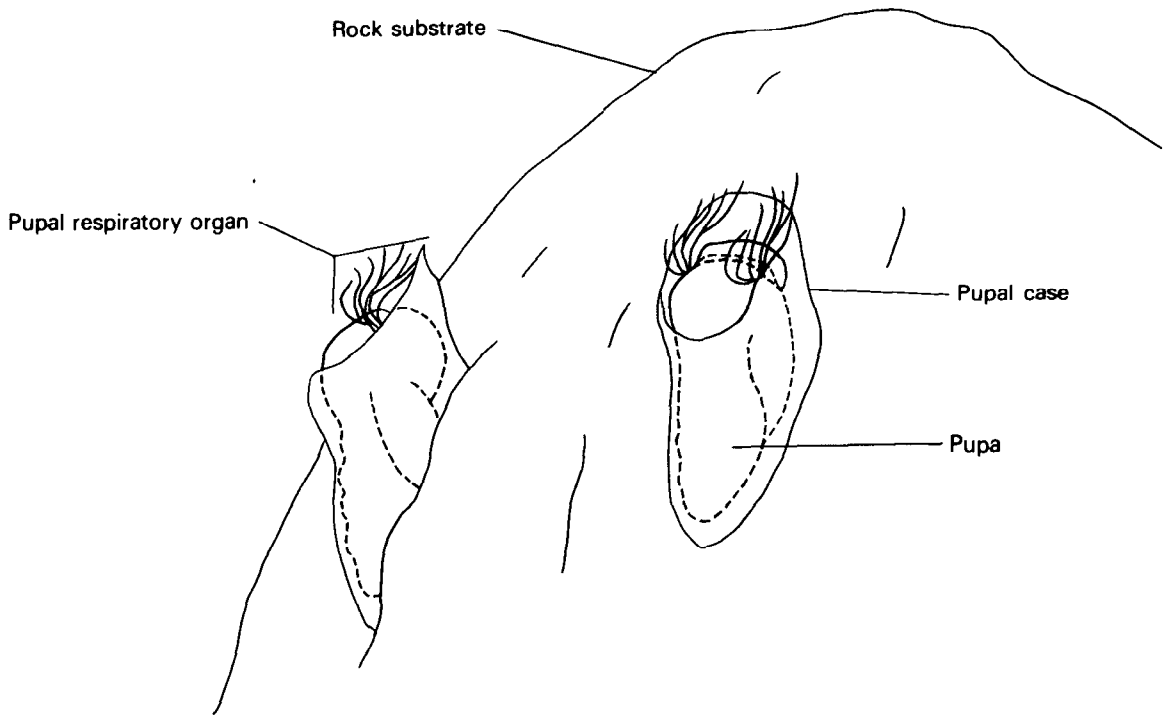


Figure 41.—One type of pupa of the Family Simuliidae enclosed in a slipperlike case attached to rocks in the water.

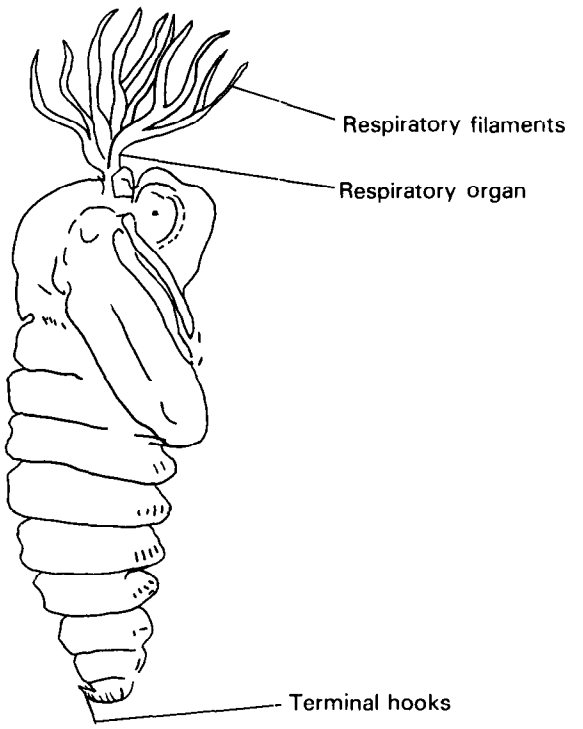


Figure 42.—Simplified features of a pupa of the Family Simuliidae showing location and arrangement of the pupal respiratory filaments.

thread-like secretion (silk) from the head prevents the larva from being swept away by the current. The larval head capsule has many features used for identification. These include the arrangement of spots on the dorsal side, relative length and color of the antennae, shape of the occipital cleft located on the ventral surface (fig. 44), and the shape and tooth pattern of the submentum (fig. 44). The shape of the secondary mouth fan (fig. 45), used to filter food particles from the water, is an additional characteristic used for identification. The fan is exposed by grasping the larva firmly near the head, ventral side up, and lifting the primary fan up and out (Sommerman, 1953).

On each side of the prothorax of a mature larva are histoblasts of the developing pupal respiratory organ (fig. 43). The number of filaments and their branching pattern are used for identification and to associate the larva with the pupa.

On the dorsal surface of the eighth abdominal segment are three simple or branched anal gills (fig. 43) that aid in respiration. These gills, which are useful for identifying genera, often are hidden in the rectal opening and may have to be exposed through dissection (Sommerman, 1953). In some genera, a pair of ventral tubercles is present just anterior to the posterior crochet ring (fig. 43).

Except for very small or mutilated specimens, most larvae and the pupae can be identified using a dissecting microscope without preparing a mount. Microscope slide mounts of the head region, however, are especially useful in identification of larvae to the species level.

1. Applications

The method is suitable for all immature Simuliidae.

2. Summary of method

The immature simuliids in a sample are examined and identified as precisely as possible without dissection or mounting. If necessary, dissection is performed and slide mounts are made. The taxa and numbers of individuals within each taxon are recorded and reported as a percentage of the total benthic-invertebrate population or reported in other ways appropriate to the study objectives.

3. Interferences

During slide preparation, overheating the larvae in 10-percent potassium hydroxide may result in brittleness, excessive transparency, or digestion of materials. The

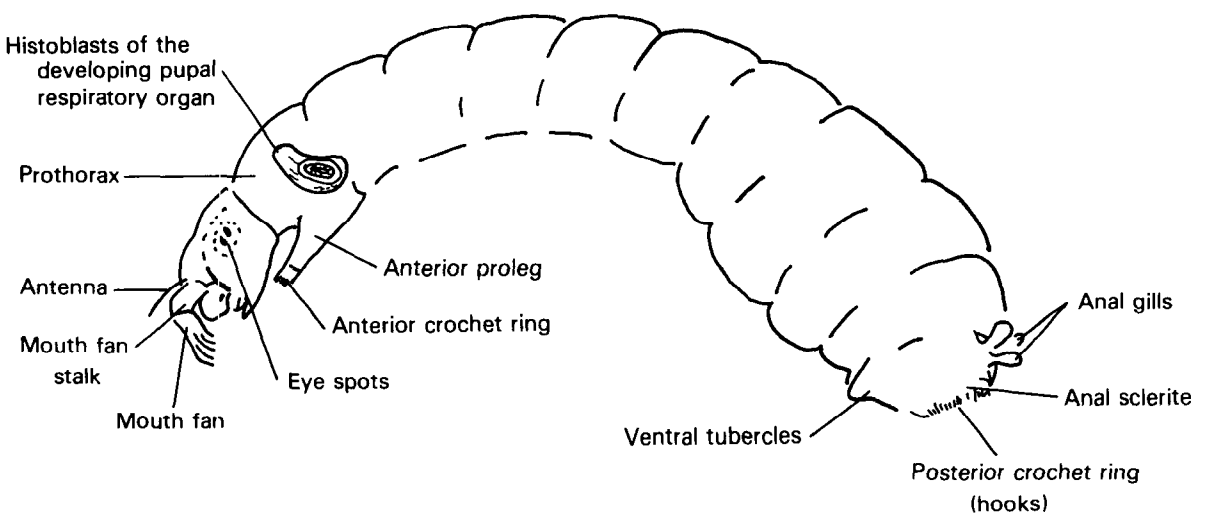


Figure 43.—Mature larva of the Family Simuliidae, simplified, showing most of the important external features needed for identification.

antennae are especially difficult to see if the specimen is overheated.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Cover glasses*, circular, No. 1 or 2, 12-mm diameter.

4.2 *Crucibles*, high-form, porcelain, 10-mL capacity.

4.3 *Forceps*, blunt curved tips, and *microforceps*, fine-tipped.

4.4 *Hotplate*, electric.

4.5 *Labels*, for microscope slides. When many slides are prepared, information about the source of the sample can be typed on sheets of paper, photocopied and reduced one-half or two-thirds in size, cut out, and glued onto slides using white glue, or equivalent. *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.6 *Marking pen*, permanent, waterproof, for labeling slides.

4.7 *Microscope, compound*, preferably having differential interference contrast capable of 1,000× magnification.

4.8 *Microscope slides*, glass, precleaned, 25×75 mm.

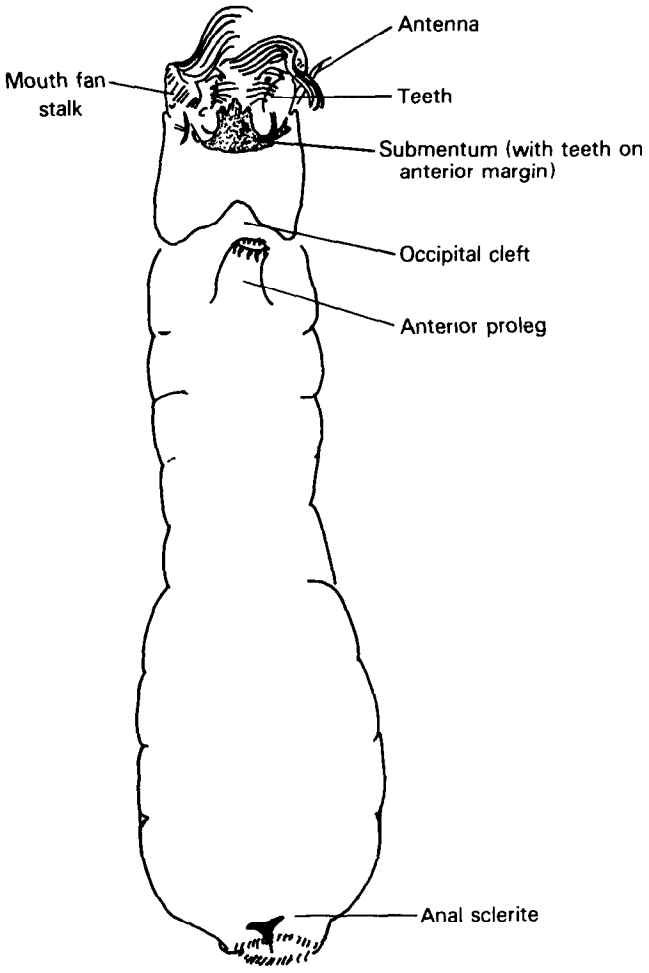


Figure 44.—A larva of the Family Simuliidae, simplified, showing the features that can be seen best after making a permanent mount.

4.9 *Needles*, for manipulating and dissecting specimens under stereomicroscope.

4.10 *Ocular micrometer*, graduated to 5 μm.

4.11 *Stereoscopic zoom microscope* (dissecting), capable of 80× magnification.

4.12 *Vials*, 4 mL, and poly seal screw lids.

4.13 *Watchglass*, Syracuse type.

4.14 *White glue.*

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Acetic acid*, glacial.

5.2 *Distilled or deionized water.*

5.3 *Fingernail polish*, clear.

5.4 *Glycerin.*

5.5 *Mounting medium*, CMC-10, or prepare medium as follows: In 50 mL distilled water, dissolve 30 g Gum arabic (amorphic), 200 g chloral hydrate, and 20 mL glycerin. Completely dissolve each solid ingredient before adding succeeding reagents. Filter final mixture through clean cheese cloth.

5.6 *Potassium hydroxide solution*, 10 percent. Dissolve 10 g potassium hydroxide (KOH) pellets in 100 mL distilled water.

5.7 *Preservative solutions*. Samples may be preserved in

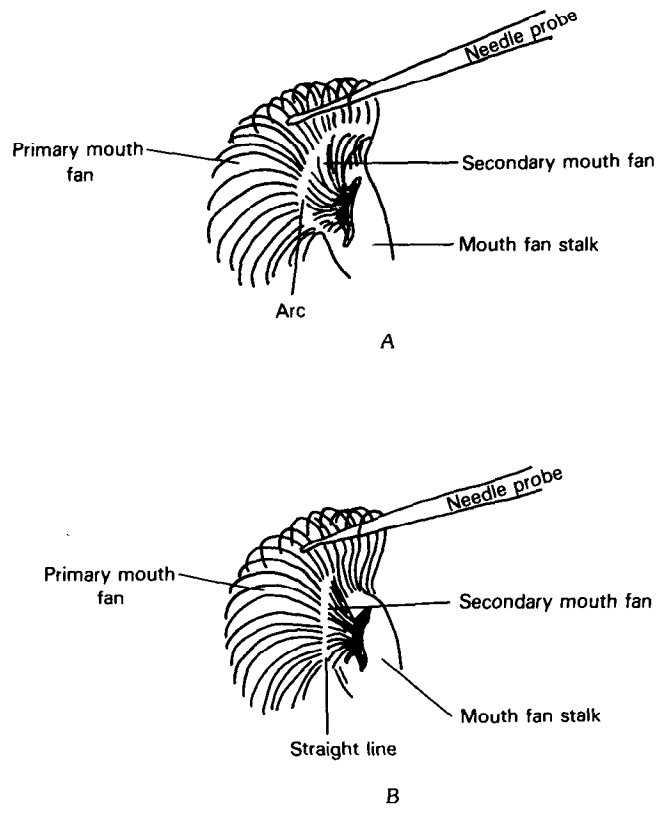


Figure 45.—Simuliidae larval mouth fans showing the two basic types of secondary fans, tips of the expanded secondary fan falling into: (*A*) an arc, and (*B*) a straight line.

70-percent ethyl alcohol or 70-percent isopropyl alcohol. A mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.7.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.7.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.7.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

6. Analysis

Usually, time does not permit mounting all the simuliids in a large sample, so the results from a subsample are used to calculate the distribution of taxa and number of individuals in the original sample. The size of the subsample for microscopic examination will depend on the size of the original sample, the number of visually distinct groups (see 6.2), and the study objectives.

6.1 Separate the pupae from the larvae and identify using a dissecting microscope. Identification of pupae is based primarily on the number and arrangement of respiratory filaments on the thorax. Slide mounts of pupae are not necessary because the filaments are clearly visible.

6.2 Using a dissecting microscope that has 7× or 20× magnification, separate the total larval group into subgroups on the basis of general external characteristics (for example, body color, presence or absence of ventral tubercles, color and length of antennae, size and shape of occipital cleft, and number and type of anal gills). Experience using taxonomic keys will aid in the selection of diagnostic characteristics for separating the subgroups.

6.3 Randomly select representatives of each subgroup for detailed microscopic examination and possible mounting. For small subgroups that have 10 or fewer individuals, select 5, or at least 50 percent. For larger subgroups, the subsampling should be by stratified random sampling and cluster or two-stage sampling. Store the remaining specimens in vials of 70-percent ethyl alcohol containing one or two drops of glycerin.

6.4 Place the selected larvae in a dish of 70-percent ethyl alcohol and examine using a stereoscopic microscope at a magnification of 10× to 70×. Identify the specimens using an appropriate taxonomic key. Examples of useful keys are Stone (1952), Sommerman (1953), Stone and Jamnback (1955), and Peterson (1970, 1978, 1981).

6.5 In mature Simuliidae larvae, the histoblasts of the developing pupal respiratory filaments are well developed and can be used to identify the larvae with the pupal stage. The filaments are important key characteristics. Dissect them by piercing the integument around the entire filament, lift the filament, and cut it at the base. Record the number and pattern of the filament branches. Mount the filaments in a drop of mounting medium on a glass slide. Place a cover glass on the drop, and press firmly using a pair of curved-tip, blunt forceps.

If more information is needed to complete the larval identification, proceed to 6.6 through 6.10, which describe preparation of microscope slide mounts. Mounts facilitate identification of many small larvae by enabling the examination for submental teeth, mouth fan rays, and anal sclerites (fig. 45). Before mounting, be sure to record the important characteristics of the head specified in the keys, such as the anal gills, occipital cleft, ventral tubercles, and antennae, because they may be distorted when mounted.

6.6 Select eight larvae, and rinse each one in distilled water for 2 or 3 minutes. A Syracuse watchglass is a convenient vessel.

6.7 Place the larva in a high-form porcelain crucible containing 10-percent KOH, and heat on a hotplate for 8 to 15 minutes or until the body is noticeably lighter in color.

6.8 Rinse the larva in distilled water (Note 1) for 2 to 3 minutes, and rinse with 95-percent ethyl alcohol for at least 3 minutes to remove the residual water and KOH.

Note 1: Glacial acetic acid can be used to remove the KOH.

6.9 Place each larva in a drop of mounting medium on a clean glass slide and, using needles, position the specimen ventral side up. Place a circular cover glass on the preparation and press firmly using a pair of curved-tip, blunt forceps. Ensure that the larva remains ventral side up while pressing and that the antennae are clearly visible. Check the slide for clarity of diagnostic characteristics using a compound microscope. Allow preparation to dry for 1 week at room temperature, keeping the slide horizontal.

6.10 Specimens may dry after 2 or 3 years in the mounting medium unless the edges of the cover glass are sealed. To make the preparations more permanent, ring the slide by coating the edges of the cover glass and any exposed mounting medium with clear fingernail polish.

7. Calculations

7.1 When only part of the total sample of Simuliidae larvae is mounted and identified, project the results from the subsample to the total number of Simuliidae in the original sample:

$$\text{Total number of individuals of a particular taxon in sample} = \frac{\text{Number of individuals of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of individuals of a particular taxon}}{\text{Total number of individuals of all taxa}} \times 100.$$

8. Reporting of results

Report the number of taxa present, the number and percentage of individuals in each taxon in the sample, and the method of collection.

9. Precision

No numerical precision data are available.

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Permanent- and semipermanent-slide method for aquatic Acari

(B-5240-85)

Parameter and Code: Not Applicable

Water mites of the Order Acarina are found worldwide in almost all types of aquatic habitats, from the hot springs of Yellowstone National Park to the cold tundra pools of Alaska, and from swift, turbulent mountain streams to quiet lakes and stagnant ponds. Most species live in freshwater, although a few are strictly marine. Some species are subterranean. The adults and nymphs generally are free-living and predaceous, while the larvae primarily are parasitic on the immature and adult stages of Diptera, Hemiptera, Odonata, Plecoptera, and other aquatic and semiaquatic insects. The larvae also are known to parasitize the gills of crabs and mussels.

Water mites have little economic significance other than being food for fishes, such as the brook and rainbow trout (Marshall, 1933); however, this little-known group of arthropods may have unrecognized economic importance as a biological control agent of mosquitoes and other biting insects. Uchida and Miyazaki (1935) reported that an *Anopheles* mosquito infested with five or more mites cannot be induced to bite, thus interrupting the life cycle that is dependent on a blood meal. Abdel-Malek (1948) reported that *Aedes* adults infested with water mites produced fewer eggs than uninfected individuals.

Water mites may prove important in water-quality studies because of their acute sensitivity to environmental stress (Young, 1969) and their species and even their generic specificity for particular habitats. The water-mite fauna found in a cold mountain stream is distinctively different from the fauna of a pond or lake or the fauna of a hot spring.

A water mite has four stages in its life cycle—egg, larva, nymph, and adult. The larva, the smallest stage, has three pairs of legs instead of four pairs as in the nymph and the adult stages. The nymph is larger than the larva and commonly is brightly colored with shades of red and orange, especially in stillwater forms. Stream mites frequently are a dull brown or greenish brown.

The adult water mite is ovoid to globular in shape and has an unsegmented, fused cephalothorax and abdomen. The sexes are separate. The dorsum may be thin and leathery or may have sclerotized plates (fig. 46). The legs have short bristles and long swimming hairs, particularly in the pond and lake forms. The nymph differs from the adult by having an incomplete genital field; that is, it lacks a genital opening and has fewer genital acetabula (fig. 46).

The anterior end of the body has the mouth region or gnathosoma (fig. 46), which sometimes is lengthened anteriorly into a rostrum. At the base of the gnathosoma are two pairs of mouth parts that are key characteristics for identification, a pair of chelicerae (mandibles) and a pair of palps. The palps consist of five segments—P_1 through P_5 (fig. 47)—that may have a number of setae and spines and terminate in simple or scissorlike claws.

The coxal parts of the legs, called epimeres (fig. 46), are on the underside or venter of the mite. There are four pairs of epimeres that vary in shape, position, and degree of fusion or separation. The genital field, consisting of a number of acetabula and a genital opening, is either between or behind the fourth epimere, or on the posterior margin of the venter.

Other diagnostic characteristics on the venter are three pairs of epimeroglandularia, each of which consists of a gland pore and a hair or seta. Epimeroglandularia I usually is found between epimere II and III; epimeroglandularia II is variable in position, but often is lateral to the genital opening; and epimeroglandularia III is behind epimere IV. The configura-

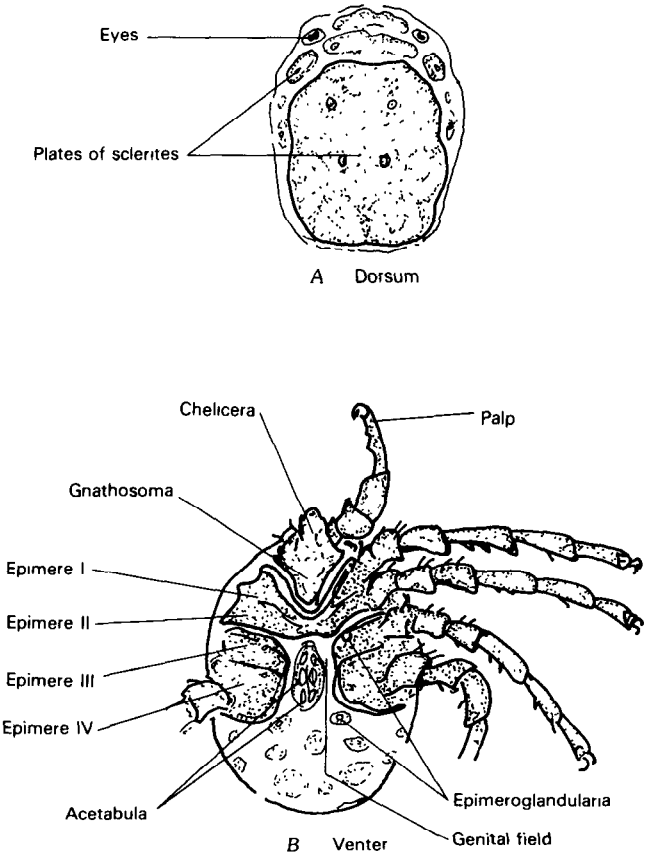

Figure 46.—Dorsal (*A*) and ventral (*B*) views of an adult water mite showing important morphological features used for identification.

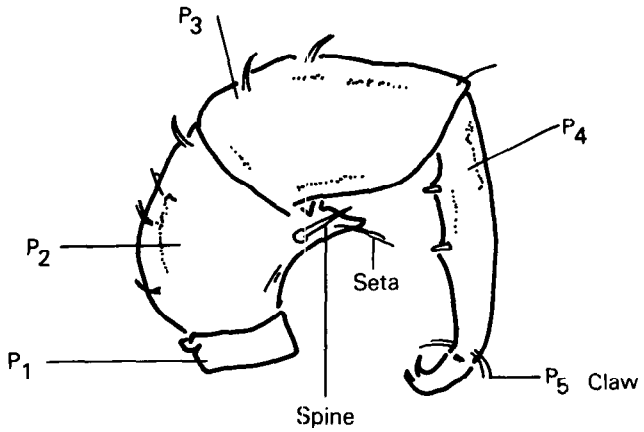

Figure 47.—Five-segmented palp (P_n) of a water mite.

tion of the epimeres, the number and arrangement of the acetabula in the genital field, and the relative position of the epimeroglandularia are important characteristics used in the identification of water mites.

Minimal information about water mites of streams exists. There are scattered descriptions of stream mites, but no single work exists that can be used for identifying them. In contrast, the water mites of ponds and lakes have been fairly well studied. Since the early 1900's, a few descriptive papers on North American water mites have appeared, particularly by researchers such as Marshall (1940, 1943), Cook (1954a,b, 1974), Crowell (1960), and Krantz (1975). Mitchell's (1954) checklist is a valuable source of information about reported American water-mite species and the relevant literature. To collect specifically for water mites, use the procedures described by Cook and Mitchell (1952).

To adequately identify water mites, mounts must be made for microscopic examination. The method described in this section is a modification of the double cover-glass glycerin method developed by Mitchell and Cook (1952) and Cook (1974).

1. Applications

This method is suitable for freshwater and marine mites, in the adult or nymph stage, that have been preserved in alcohol.

2. Summary of method

The water mites in a sample are dissected, cleared, and permanent-slide mounts are made for microscopic examination and identification. The kinds of taxa and the number of individuals in each taxon are recorded and reported as a percentage of the benthic-invertebrate population or reported in other ways appropriate to the study objectives.

3. Interferences

Failure to remove or digest the body contents of water mites will result in obscured mounts. Prolonged soaking in potassium hydroxide may damage the cuticle of mites. Unless the more time-consuming method is used, mounts will continue to clear and fade for a few days after slide preparation is complete, making specific identification difficult and sometimes impossible.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Cover glasses*, circular, No. 1, 12 mm, and cover glasses, circular, No. 1, 22 mm.

4.2 *Forceps*, blunt curved tips, and *microforceps*, fine-tipped.

4.3 *Hotplate*, electric, or *slide warmer.*

4.4 *Labels*, for microscope slides. When many slides are prepared, information about the source of the sample can be typed on sheets of paper, photocopied and reduced one-half or two-thirds in size, cut out, and glued onto slides using white glue, or equivalent. *Labels, waterproof*, or labels may be cut from sheets of plastic paper.

4.5 *Marking pen*, permanent, waterproof.

4.6 *Microscalpel*, capable of dissecting a specimen, 0.75 mm in diameter. A No. 1 insect pin, mounted on a wooden applicator stick and shaped into a microscalpel using a fine hone or emery cloth and a dissecting microscope, is satisfactory (Cook, 1974).

4.7 *Microscope, compound*, preferably having differential interference contrast capable of 1,000× magnification.

4.8 *Microscope slides*, glass, precleaned, 25×75 mm.

4.9 *Needles, pins, or probes*, for manipulating specimens under a stereomicroscope.

4.10 *Oven.*

4.11 *Spot plates*, white porcelain.

4.12 *Stereoscopic zoom microscope* (dissecting), 30× to 70× magnification.

4.13 *Vials*, 4 mL, and poly seal screw lids.

4.14 *Watchglass*, Syracuse-type.

4.15 *White glue.*

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Canada balsam*, grade A.

5.2 *Corrosive lactophenol.* Add 50 mL lactic acid to 25 mL distilled water. Add 25 g phenol crystals and dissolve completely.

5.3 *Distilled or deionized water.*

5.4 *Fingernail polish*, clear.

5.5 *Glycerin.*

5.6 *Glycerin jelly.* Melt jelly in a dropper bottle or vial emersed in a beaker of hot water. Heat water just enough to liquefy the jelly.

5.7 *Mounting medium*, CMC-10, or prepare medium as follows: In 50 mL distilled water, dissolve 30 g Gum arabic (amorphic), 200 g chloral hydrate, and 20 mL glycerin. Completely dissolve each solid ingredient before adding succeeding reagents. Filter final mixture through clean cheese cloth.

5.8 *Potassium hydroxide solution*, 10 percent. Dissolve 10 g potassium hydroxide (KOH) pellets in 100 mL distilled water.

5.9 *Preservative solutions.* Samples may be preserved in 70-percent ethyl alcohol or 70-percent isopropyl alcohol. A

mixture of 70-percent ethyl alcohol and 5-percent glycerin is preferred for permanent storage. Prepare as follows:

5.9.1 *Ethyl alcohol.* Dilute 70 mL 95-percent alcohol to 95 mL using distilled water.

5.9.2 *Ethyl alcohol and 5-percent glycerin.* Dilute 70 mL 95-percent alcohol to 100 mL using 25 mL distilled water and 5 mL glycerin.

5.9.3 *Isopropyl alcohol.* Dilute 70 mL concentrated isopropyl alcohol to 100 mL using distilled water.

6. Analysis

For samples containing few water mites, prepare mounts of all individuals. If the numbers are large, separate the mites into distinct groups (see 6.1) and take a subsample of each group (see 6.2). Use the results from the subsample to calculate the distribution of taxa and individuals in the original sample.

6.1 Using a dissecting microscope with 30× to 70× magnification, separate the water mites in a sample into groups on the basis of general external characteristics. Important characteristics include color, texture of the dorsum (for example, covered by a shield, small sclerites, or leathery), epimere configuration, number and arrangement of the acetabula, and position of the genital field (fig. 46).

6.2 Proceed to 6.3 if all water mites will be mounted. In large samples, randomly select representatives of each group for mounting on slides for microscopic examination. Subsampling should be done by stratified random sampling and cluster or two-stage sampling. Store remaining mites in vials of 70-percent ethyl alcohol containing one or two drops of glycerin.

6.3 Place the specimen to be examined in a watchglass containing 70-percent ethyl alcohol. Using a dissecting microscope, microscalpel, and fine-tipped microforceps, separate the dorsum from the venter, leaving a small section of the lateral body wall intact (fig. 48). The intact body wall prevents body parts and appendages from being lost. In large specimens from which the body contents can be removed using the tip of a needle, omit 6.4 and 6.5 for clearing, and proceed to 6.6 or 6.20. If the specimen is too small for dissection, pierce the body wall in the posterio-lateral area to facilitate the clearing process.

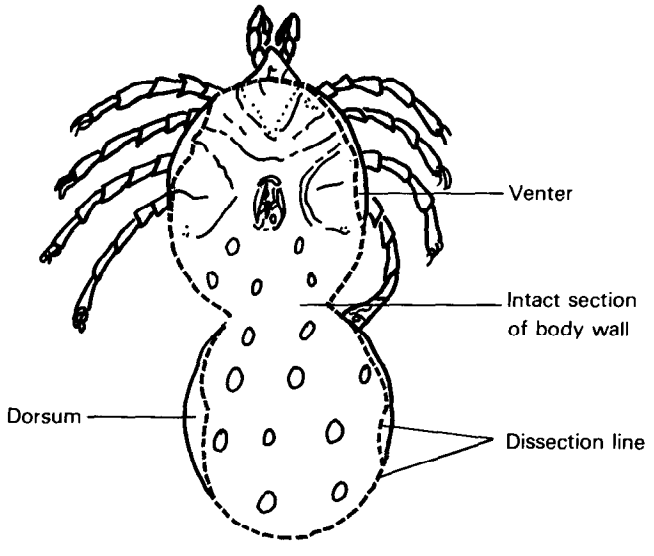

Figure 48.—A water mite showing the dorsum separated from the venter, leaving a small section of the lateral body wall intact (see 6.3).

6.4 Clear the specimen for 24 to 48 hours in a vial containing the corrosive lactophenol. Prolonged clearing has minimal damaging effect. If the specimen has a particularly hard cuticle, clear in 10-percent KOH for 1 to 2 hours. Care must be taken to avoid damage to the cuticle by prolonged soaking in KOH.

6.5 Remove the lactophenol or KOH corrosive by rinsing the specimen in three to four changes of distilled water (Note 1) followed by 70-percent ethyl alcohol.

Note 1: Two different methods of slide preparation are described based on the quality of the resulting mounts for taxonomic identification. The method described in 6.6 through 6.19 is more time consuming, but results in longer lasting slides suitable for species identification. The quicker, optional method described in 6.20 through 6.22 results in slides adequate for identification to family or genus. Selection of the method should be based on study objectives.

6.6 Transfer the specimen to glycerin. With weakly sclerotized specimens, distortion sometimes occurs when transferring directly to glycerin. For such specimens, proceed to 6.7 and 6.8.

6.7 Transfer the specimen to a depression in a spot plate containing two or three drops of alcohol-glycerin solution.

6.8 Place the spot plate and water mite in an oven at 55 °C for 30 to 40 minutes to evaporate the alcohol, leaving the mite in the glycerin.

6.9 Lift the specimen from the glycerin using the tip of a needle, and place on a 12-mm diameter circular cover glass.

6.10 Using a dissecting microscope, microforceps, and needle, separate the palps from the body by dissecting one palp from the gnathosoma or by removing the entire gnathosoma and palps. The dorsum may be severed from the venter. In very small specimens for which dissection is difficult, leave the specimen intact with the venter facing upward.

6.11 Arrange the parts on the cover glass so the original exterior surface of the venter and the dorsum faces upward, and the palps can be viewed as shown in figure 47.

6.12 Place a drop of melted glycerin jelly on the 12-mm cover glass and specimen.

6.13 Move the parts into final position and place a 22-mm circular cover glass on the smaller cover glass, jelly, and specimen.

6.14 Press large cover glass gently using curved-tip, blunt forceps to spread jelly evenly to edges of smaller cover glass, turn preparation over with smaller cover glass up, and continue pressing smaller cover glass enabling excess glycerin jelly to ooze from the edges.

6.15 Set preparation aside for at least 15 minutes to allow the glycerin jelly to set.

6.16 Place one drop of Canada balsam on a clean glass microscope slide, and place the double cover-glass preparation, 12-mm cover glass down, on the drop of balsam (fig. 49). Press lightly. If bubbles are present in the balsam under the cover glass, they may be removed by warming the slide preparation at 45 °C on a hotplate or on a slide warmer.

6.17 Label slide, using waterproof ink, and record the date, site, method of collection, identification number, or other information pertinent to the study.

6.18 Identify water mites using a compound microscope and appropriate taxonomic keys. Examples of keys for the nonspecialist are Newell (1959), Cook (1974), and Pennak (1978).

6.19 Allow slides to air-dry for at least 2 months before storing on edge.

6.20 *Optional method.* Place the specimen in a small drop of mounting medium on a clean glass microscope slide. Using a dissecting microscope, microforceps, and needle, dissect the specimen and arrange the parts as in 6.10 and 6.11. Ensure that the parts are pushed well into the medium and against the slide to prevent them from drifting away when the cover glass is applied.

6.21 Place a 12-mm circular cover glass on the drop of mounting medium containing the specimen, and press cover glass gently using curved-tip, blunt forceps. Allow preparation to dry for 1 week at room temperature, keeping the slide horizontal.

6.22 Specimens may dry after 2 or 3 years in the mounting medium unless the edges of the cover glass are sealed. To make the preparations more permanent, ring the slide by coating the edges of the cover glass and any exposed mounting medium with clear fingernail polish.

7. Calculations

7.1 When only part of the total sample of Acari is mounted and identified, project the results from those mounted to the total number of specimens:

$$\text{Total number of individuals of a particular taxon in sample} = \frac{\text{Number of individuals of the taxon in subsample}}{\text{Fraction of total sample in subsample}}.$$

7.2 Percent composition in sample

$$= \frac{\text{Number of individuals of a particular taxon}}{\text{Total number of individuals of all taxa}} \times 100.$$

8. Reporting of results

Report the number of taxa present, the number and percentage of individuals in each taxon in the sample, and the method of collection.

9. Precision

No numerical precision data are available.

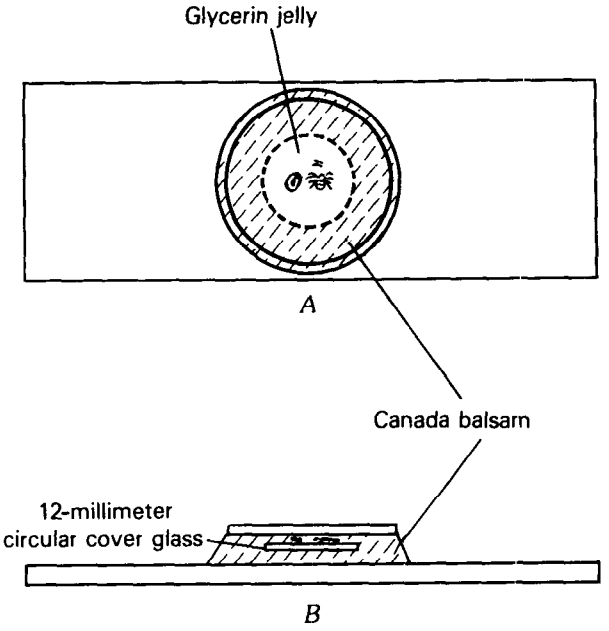

Figure 49.—Top (*A*) and side (*B*) views of the double cover-glass technique for mounting aquatic Acari (modified from Mitchell and Cook, 1952).

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