

Chapter 15

Macroinvertebrates

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15.1 INTRODUCTION

Macroinvertebrates are virtually ubiquitous in the streams and rivers of the world. Only the most harsh, fleetingly temporary, or grossly polluted lotic environments do not contain some representatives of this diverse and ecologically important group of organisms. By convention, the term “macro” refers to invertebrate fauna retained by a 500-µm mesh net or sieve. However, the early life stages of many macroinvertebrates pass through mesh openings of this size. Yet, these early stages are important to understanding species-specific life histories, trophic relations, secondary production, and a multitude of other ecological relationships and thus, these smaller sized individuals are often included as part of the “macrobenthos.” Because of this need to include small, early instars of the macroinvertebrates, there is a general trend among stream ecologists to use collecting methods employing finer meshed collecting nets (e.g., 125–250 µm) in studies where there is a need to account for these early stages. Although organisms passing through a 500-µm net, but retained by a 40-µm net, are considered meiofauna (see Chapter 14), stream ecologists studying macroinvertebrates, especially aquatic insects, have tended to include the early life stages of organisms considered “macro” in their mature stages even though they are technically smaller than the 500-µm criteria. Another convention is that the immature, larval stages of the hemimetabolous insects are typically referred to as nymphs or naiads, depending on the order they are in, whereas those for holometabolous insects are referred to as larvae.

Streams and rivers contain a remarkable diversity of macroinvertebrates. In many lotic environments, the macroinvertebrate community consists of several hundred species from numerous phyla (e.g., Konrad et al., 2008; Resh, 2008; Astorga et al., 2011; Demars et al., 2012) including arthropods (insects, mites, scuds, and crayfish), mollusks (snails, limpets, mussels, and clams), annelids (segmented worms and leeches), nematodes (roundworms), and turbellarians (flatworms). Most stream macroinvertebrate species are benthic; that is, they are associated with surfaces of the channel bottom (e.g., bedrock, cobble, and finer sediments) or other stable surfaces (e.g., fallen trees, snags, roots, and submerged or emergent aquatic vegetation) rather than being routinely free-swimming. However, many of the swimming insects found in ponds (e.g., the water boatmen and other aquatic members of the insect order Hemiptera) or even in quiescent stream pools (e.g., diving beetles) are often the most conspicuous aquatic insects observed at the water surface by visitors to streamside habitats.

In large, alluvial gravel-bed streams and rivers, the hyporheic zone (see Chapter 8) often contains strata of preferential flow characterized by large interstitial space between sorted cobble layers. Numerous species of stoneflies and other invertebrates spend the early life history stages in the hyporheic zone of stream and river floodplains. Nymphs of these insects return to the channel environment to emerge as adults and reproduce (Stanford and Gaufin, 1974; Stanford et al., 1994) while other species may remain in the hyporheic zone (Jones and Mulholland, 1999).

Partly because of their importance within the stream community as a fundamental link in the food web between organic matter resources (e.g., leaf litter, algae, and detritus) and fishes, and partly because of their diversity and ubiquity, the study of macroinvertebrates has been (Hynes, 1970; Cummins, 1974; Allan, 1995), and will continue to be (Moore and Schindler, 2008; Sundermann et al., 2011; Giersch et al., 2015), a central part of stream ecology.

Earlier chapters within this book have focused on the multitude of interactive physical, chemical, and biological variables that constitute the stream ecosystem. For example, geology, climate, and other landscape features (Chapters 1 and 2) directly affect hydrologic patterns (Chapter 3), and the movement and storage of inorganic and organic materials (Chapter 5). Later

chapters addressing nutrients and the downstream transport of solutes (Chapters 30–32) are affected by channel and substratum complexity, the interactions of ground and surface waters (Chapter 8), and by the stream biota. Likewise, interactions among the stream channel, hyporheic zone, and riparian floodplains are important features in the structure and function of the entire stream corridor (Ward, 1997; Stanford et al., 2005). Streams and rivers are critically important features for Nature because they disproportionately create high diversity of habitats, concentrate nutrients for growth, and provide corridors of connectivity linking populations that would otherwise become isolated. More than just river channels carrying water off the landscape, streams and rivers are not only used by aquatic species, but are essential to the life requirements of a wide variety of avian and terrestrial species as well affecting biodiversity from microbes to grizzly bears (Hauer et al., 2016). Stream macroinvertebrates are a significant component of both ecosystem structure and function as they play critical roles in the ecosystem, from biodiversity and bioproduction to trophic relations and energy and nutrient transfer processes.

15.1.1 Phylogeny and Adaptations

The origin of stream macroinvertebrates includes groups that are terrestrially derived (e.g., the insects) and groups that are marine in origin (e.g., mollusks and crustaceans). Of the various taxonomic groups that comprise the stream macroinvertebrate community, no group has been studied more than the aquatic insects. Not only are the aquatic insects extremely diverse, both taxonomically and functionally, but they also are frequently the most abundant large organisms collected in stream benthic samples. For example, 13 orders have aquatic insect representatives in North America (Merritt et al., 2008a,b), although only five of these are composed strictly of aquatic species (i.e., species that have at least one life-history stage that is obligatorily aquatic)—the dragonflies and damselflies (Odonata), the stoneflies (Plecoptera), the mayflies (Ephemeroptera), the caddisflies (Trichoptera), and the hellgrammies (Megaloptera). Although the remaining eight orders have primarily terrestrial inhabitants, several of these orders exhibit high species richness (often with thousands of species) in aquatic habitats. For example, the beetles (Coleoptera) and true flies (Diptera) each contain more aquatic species than are found among any of the completely aquatic orders.

The phylogeny of aquatic insects is part of what makes these organisms so interesting. No single line of evolution resulted in aquatic insects; rather insects invaded the freshwater environment many different times and in many different ways (Will and Resh, 2008). As a result, problems of living in the stream environment, such as how to obtain oxygen or remain in a fixed position, have had to be solved repeatedly. Consequently, the mechanisms developed to overcome various physical limiting factors involve a variety of different approaches and morphological adaptations. For example, some lotic species have developed structures to obtain oxygen from the atmosphere (analogous to snorkeling), others use the temporary storage of an air bubble (analogous to SCUBA diving), a few species use respiratory pigments (analogous to vertebrate hemoglobin), and many species have developed tracheal gills for obtaining dissolved oxygen directly from the water (Resh et al., 2008). Likewise, morphological adaptations for existence in a running water environment include sclerotized projections along trailing edges of legs and body to form hydrofoils that press the organism onto the substratum, streamlining of body shape to offer reduced resistance while swimming, suckers and modified gills to attach to smooth surfaces, and leg and anal hooks to attach to a variety of surfaces, to name but a few (Will and Resh, 2008). The Trichoptera, Lepidoptera, and Diptera also use silk in a myriad of ways for attachment (e.g., free-living caddisflies and black flies), food gathering (e.g., net-spinning caddisflies), and shelter construction (e.g., midge larvae, moth larvae, and cased caddisflies).

Life-history features that govern the reproduction and survival of lotic macroinvertebrates also show adaptations to specific characteristics of running water environments. Many stream environments are very dynamic (hydrologically, spatially, thermally, trophically, etc.), and macroinvertebrate life histories reflect this through tremendous diversity and adaptability (Butler, 1984). For example, some species are specially adapted to ephemeral streams by having dormant egg stages that hatch as they are hydrated when flow resumes (Williams, 1987). Also, closely related species that perform a similar trophic function may temporally separate growth and adult emergence within the same stream reach (Hildrew and Eddington, 1979; Hauer and Stanford, 1982a, 1986). Other life-history adaptations can be seen in the seasonal timing of larval diapause (Gray and Fisher, 1981) or pheromone release by adults for mate attraction (Resh et al., 1987). There is also considerable variation in the length of life cycles—some species may have several complete life cycles per year (multivoltine), two life cycles per year (bivoltine), one life cycle per year (univoltine), or may require two or three years to complete a life cycle (semivoltine). Specific life histories may also be very different across the geographic distribution of a species, where in one portion of its range a species may be univoltine and in another portion (generally colder) it is semivoltine. For example, the limnephilid caddisfly *Dicosmoecus gilvipes* is univoltine in coastal streams of California and Oregon (Lamberti et al., 1987) but semivoltine in mountain streams of Montana (Hauer and Stanford, 1982b).

Behavioral adaptations are evident in aquatic insects as well, and these include regulatory behaviors to increase the control that an individual exerts over its own metabolic status, foraging behavior that involves the gathering and processing

of food resources, or reproductive behavior that is responsible for the successful continuation of life into the next generation (Wiley and Kohler, 1984). For example, dispersal as *behavioral drift*, the intentional entry of benthic animals into the water column and their subsequent downstream transport, is a topic that has greatly interested stream ecologists for over three decades (Waters, 1972; Müller, 1974; Brittain and Eikeland, 1988; see also Chapter 21) and may be essential to colonization processes, the search for food, or predator avoidance.

Hydrologic processes, food resources, nutrient dynamics, riparian vegetation, and many other factors intimately affect the structure and function of stream ecosystems (Stanford et al., 2005). A fundamental characteristic of these factors is that they change along the longitudinal profile of the stream ecosystem (Vannote et al., 1980), and these factors may be affected by various anthropogenic influences (e.g., stream regulation; Poff et al., 1997; Stanford and Ward, 2001). Macroinvertebrate species composition also changes between headwaters, middle reaches, and large rivers, in response to changes in the stream environment. For example, a stream reach flowing through a deciduous forest with a dense overhanging canopy may have a large number of macroinvertebrates that specialize in feeding on leaf litter, but that same stream upon entering a meadow (and thus having an open canopy) may be dominated by species that graze on periphyton. Within functionally similar groups (e.g., those that feed on similar food resources and use similar feeding mechanisms; see Chapter 20 and Merritt et al., 2008a,b), species replacement along the river continuum is also very common. For example, among the net-spinning caddisflies (Hydropsychidae) numerous species may occur within a large river basin and be distributed in a very predictable manner along the longitudinal stream profile (Hildrew and Eddington, 1979; Hauer and Stanford, 1982a). Some species occur only in first- and second-order streams, other species replace the headwater species in third- through fifth-order middle reaches, and still other species will occur only in larger rivers (Hauer et al., 2000). Each species of hydropsychid spins a silk-thread catchnet that filters food particles from the flowing waters with different levels of efficiency (Edler and Georgian, 2004). Yet, through selection of particular habitats, food resources, and temperature regimes, these species exhibit very predictable landscape-scale distributions. This phenomenon is not restricted to the hydropsychid caddisflies, but rather occurs among the various species within trophic and phenologic groups (Hauer et al., 2000) (Fig. 15.1).

Students and others collecting stream macroinvertebrates for the first time are often amazed at both the complexity of the community and the wondrous variety of habitats in which they are found. Some species of macroinvertebrates exist exclusively in very turbulent, high-velocity waters where they use sucker discs, hooks, or silk to remain attached to the substratum. Other species occur in pools where stream current is slower and their specialized body structures permit them to move across the fine sediments that accumulate in pools and backwaters. Many species can be found in leaf packs where they are surrounded by the food they eat and still others bore under the bark and through the boles of large wood debris that has fallen into the stream or river (Merritt et al., 2008a,b, see also Chapters in Section D – Organic Matter Dynamics).

15.2 GENERAL DESIGN

Below, we describe several approaches to the study of stream macroinvertebrates. The purpose of these exercises is to expose stream researchers to various field and laboratory methods for the collection and study of macroinvertebrates. Over the past three to four decades, thousands of studies have focused on stream macroinvertebrates. Numerous detailed scholarly works dedicated to macroinvertebrate collection and analysis also exist, such as collecting and sampling (Merritt et al., 2008a,b), sampling design (Resh, 1979; Norris et al., 1992; Carter and Resh, 2001, 2013), and statistical analyses and study design (Norris, 1995; Hawkins et al., 2000). The purpose of this chapter is neither to synthesize nor replace these detailed examinations, but rather to introduce stream ecology students or researchers who have not worked previously with macroinvertebrates to this interesting and diverse group of organisms. Summaries of the biology of the different orders of aquatic insects are presented in Resh and Carde (2004, 2009).

Although the collecting methods we describe are most easily performed in wadeable, small to midsized streams, they can be adapted to larger rivers. While applying the various methods and exercises described below, note the tremendous variety of habitats and the different ways in which macroinvertebrates are adapted to use resources. The specific objectives of this chapter are to: (1) familiarize students and researchers with a variety of techniques for sampling stream macroinvertebrates, (2) describe how to preserve and process samples for laboratory examination, (3) introduce the concepts of abundance and diversity of stream macroinvertebrates, (4) examine large-scale distribution patterns, and (5) investigate microhabitat utilization and movement. We give only examples of approaches to be taken.

15.2.1 Field Sampling

Over the past several decades, many different types of sampling devices have been invented for the systematic collection of stream macroinvertebrates, yet only a few standard sampling devices are used for most studies. Stream macroinvertebrates

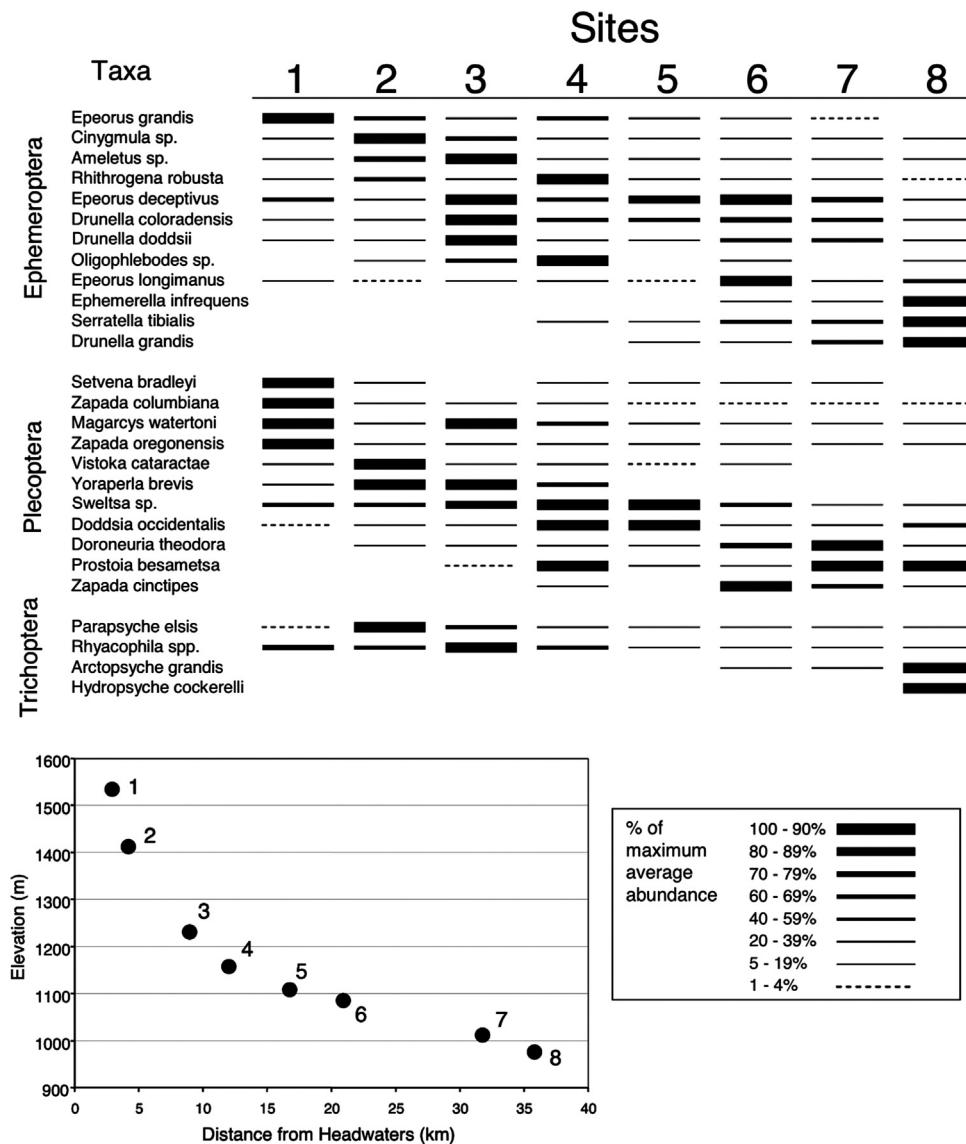


FIGURE 15.1 Frequency of macroinvertebrate fauna collected along the longitudinal and elevation stream gradient of McDonald Creek in Glacier National Park, Montana, USA. Modified from Hauer et al. (2000).

are generally collected by disturbing bottom sediments (e.g., gravel, cobble) and catching organisms in a net-held downstream. Many samplers are designed to delineate a certain area of stream bottom (e.g., 1 ft², 0.25 m², 0.5 m²). Then, by dislodging the substratum materials the benthic macroinvertebrates are disturbed and captured as they are swept into the net by the current.¹ Merritt et al. (2008a,b) provide an excellent review of various macroinvertebrate samplers used in streams and rivers.

The Surber sampler (Surber, 1937) and Hess sampler (Hess, 1941) are two standard collecting devices for stream macroinvertebrates that have been used widely in stream ecology for over 75 years. Both samplers generally are small and limited to sampling stream depths <15 cm and in streams with small substrata (i.e., sand, gravel, and very small cobble). Another standard collecting device for stream macroinvertebrates is the kicknet, so-named because of the kicking action done in front of the net. The simplest kicknet is easy to make. Using two wooden dowels or broom handles about 1.25-m long and 2–3 cm in diameter, attach a 1 m × 1 m square of 500-μm mesh Nitex netting (Fig. 15.2). In some cases, similar-meshed window screening has been used. To collect the organisms from the bottom of the stream, hold the net open by the

1. Note: A video series that demonstrates over 20 collecting techniques usable in a variety of stream habitats is available at <http://nature.berkeley.edu/reshlab/>.

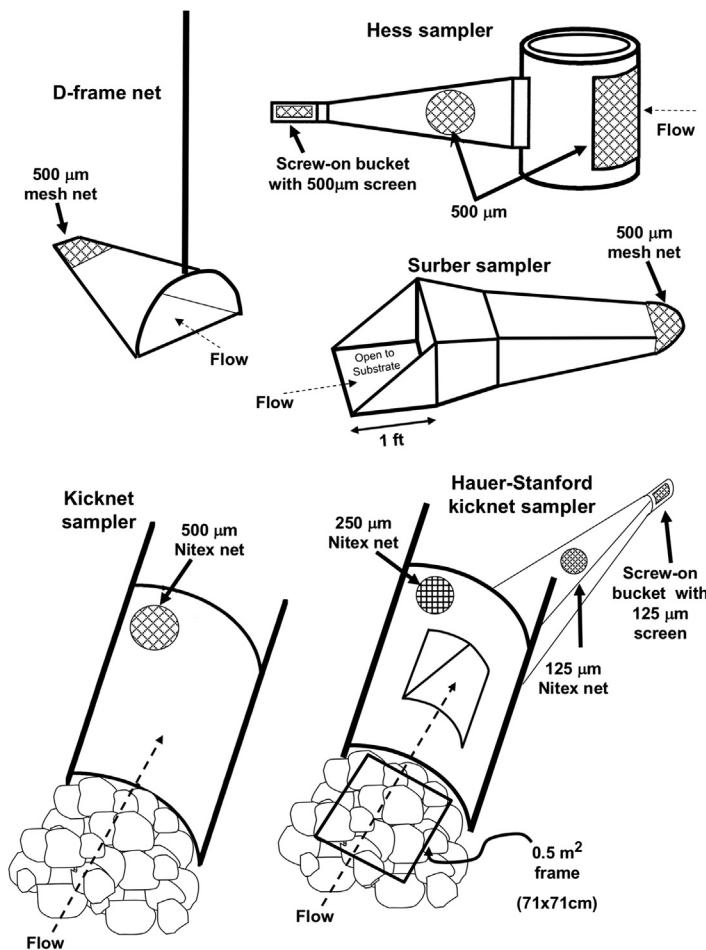


FIGURE 15.2 Illustrations of the most common sampling devices used to capture macroinvertebrates in streams with gravel and cobble-bed material.

two handles perpendicular to the flow and against the substratum. Then disturb the area in front of the net; generally this is done by moving the substratum with feet and hands. The organisms are collected from the net surface after the net is removed from the stream. This collecting net has the major drawback of often rapidly filling with material that clogs the net and results in back welling. As the net is no longer filtering water, the organisms are swept back out of the net, thus adding sampling error to the collection.

We have found that for large cobble to small boulder-size substratum (10–30 cm) the Surber sampler and Hess sampler are either too small or cannot be sealed around the bottom of the sampler, thus introducing unacceptable sampling error. When sampling this larger sediment size substratum, use a modification of the kicknet takes advantage of the kicknet's size and allows for efficient sampling of substratum that is too large for Surber or Hess samplers and more efficient than a D-frame sampler. The Hauer-Stanford kicknet (Hauer and Stanford, 1981) was designed specifically for sampling cobble sized material that is common in gravel-bed rivers, taking advantage of the size and principal of the kicknet with the filtering capacity of the Surber sampler. But, unlike the Surber, the Hauer-Stanford net can be used in deep (20–40 cm), swift (>50 cm/s) riffles and runs, which are very typical of three- to six-order streams and rivers. Operation of the Hauer-Stanford kicknet may be done with either one or two persons (Fig. 15.3). In the two-person operation, one person opens the kicknet and lowers the net base to the substratum oriented perpendicular to the stream current. The second person places a 0.5-m² frame made of 1/4- or 3/8-in. diameter steel rebar in front of the net. Then cobble from within the frame is disturbed one stone at a time, and carefully brushed and washed. The organisms from each stone are washed into the net by the current. The sample area is then vigorously disturbed by stepping into the framed area and kicking back and forth for about 30–60 s. The Hauer-Stanford kicknet can also be operated by a single individual if the current is not too strong.

Numerous sampling devices have been designed for collecting macroinvertebrates from other stream substratum types. Coring and dredging devices have been used to sample soft sediments such as sand or mud and frequently are necessary for



FIGURE 15.3 Field pictures of the Hauer-Stanford kicknet being used by (A) two persons and (B) by a single person. Note the extended net and bucket end of the kicknet sampler.

sampling in large rivers or wherever soft sediments are prevalent or where waters are deep and sampling is done from a boat. The D-frame net may be used to sample gravel or cobble substrata, soft sediments, or woody snags (Fig. 15.2), but also has limitations in the sampling error due to large numbers of individual macroinvertebrates dislodged from the sample area not being captured in the net.

After a sample is collected, the organisms are rinsed into the end of the net. At streamside, the contents of the sample are poured through a fine-meshed net or sieve of 125 µm or less, depending on original mesh size of the sampler, to remove excess water from the sample. If the sample is large it may be washed into a 20-L plastic bucket prior to transfer into the sieve. Samples that are to be returned to the laboratory should be placed into a plastic jar or ziplock bag and preserved in a 70% ethanol solution. This may be best done by adding 95% ethanol to the sample and estimating the remaining water, body fluids, and other organic matter to dilute the ethanol concentration to 70%.² Ideally, samples should be sorted within 24–48 h after collection to prevent specimens from deteriorating. In some cases, immediate live sorting may be useful, assuming samples are kept cool. After identification, stream macroinvertebrates that are intended for long-term storage should be curated in a glass patent-lip vial with a neoprene stopper, clean 70% ethanol, and a proper label.

By using a Surber, Hess, or Hauer-Stanford kicknet sampler (or some other sampling device with a defined area), quantitative samples may be collected from a known area and in a standardized fashion to obtain sample replicates. From these replicates, a sample mean and variance can be calculated to estimate the population size and variability. Quantitative sampling is necessary for most ecological investigations and involves a variety of decisions, including choice of sampling sites, depth of penetration of sampling into the substrata, frequency of sampling, and other decisions. Bias (i.e., the lack of congruence between what is in a sample and what actually occurred in the sample area) can result from factors related to the characteristics of the sampler (e.g., backwashing of the sample from a clogged net), the organisms being sampled (e.g., tight attachment to substrata, movements to avoid being caught), and inconsistency among the users of the sampling devices (Resh, 1979). The nonrandom distributions of most stream macroinvertebrate populations may require that large numbers of samples be collected in quantitative studies.

15.2.2 Laboratory Procedures

Sorting, which is done in the laboratory, involves the separation of the benthic macroinvertebrates from the inorganic and organic matter collected with the macroinvertebrate sample. Sorting can be time-consuming, but using sieves to separate out larger particles, dyes to stain macroinvertebrates, adding sugar to change the specific gravity of the liquid and thus “float-off” organisms, and subsampling of very large samples can all greatly reduce sorting time and effort. Large organisms can be easily seen and sorted without the aid of magnification; however, small species (e.g., microcaddisflies,

2. Note: Traditionally, samples were preserved with 5% formalin solution or Kahle's fluid (28% ethanol, 11% formalin, 2% glacial acetic acid, and 59% water). Although formalin and Kahle's are very effective preservatives, they are hazardous to use and difficult to dispose of after use. Formalin is a known carcinogen and should be avoided.

midge larvae) and early instars of even the large species of macroinvertebrates require scrutiny under a good dissecting microscope.

After samples have been sorted, organisms must be identified and counted. The results are then analyzed by various procedures depending on the research questions and the accompanying experimental design. By following simple keys based on distinguishing morphological characteristics, it is relatively easy to identify macroinvertebrates to the family level. However, distinguishing macroinvertebrates at the generic or species level generally requires substantial training. What level of identification is required? The answer depends on the objective of the study (see discussion in [Lenat and Resh, 2001](#)). We have included a simple flow-key to the more common stream macroinvertebrates ([Appendix 15.1](#)). Detailed keys to the families of North American freshwater invertebrates ([Thorp and Covich, 2014](#)), and to the genera of North American aquatic insects ([Merritt et al., 2008a](#)) and non-insects ([Pennak, 1989](#)), are excellent starting points for more detailed identification of stream macroinvertebrates. [McCafferty \(1981\)](#) has excellent illustrations of aquatic insects to the family level. There are also many field guides available for different areas, identification information that can be viewed on the internet, and new identification guides to the fauna of other continents. There also are several sources for identification of macroinvertebrates specifically designed for anglers and citizen science programs (e.g., [Knopp and Cormier, 1997](#); [Rosenbauer, 2014](#)).

The availability of species-level keys are usually confined to a single genus (e.g., [Szczytko and Stewart, 1979](#)) or region (e.g., [Nimmo, 1971](#); [Baumann et al., 1977](#); [Ward and Kondratieff, 1992](#)) and are generally available as journal publications or guide books. Detailed, species-specific identification may require consultation with specialists and occasionally rearing of aquatic insects to the adult stage. Available keys for the North American fauna are listed at the end of the different order chapters in [Merritt et al. \(2008a\)](#).

15.3 SPECIFIC METHODS

15.3.1 Basic Method 1: Distributions and Habitat Relationships

If you carefully examine a reach of stream, you will discover that many populations of lotic macroinvertebrates are not distributed uniformly throughout the reach. Rather, species tend to be found in particular hydrogeomorphic habitats (see Chapters 4 and 5). Habitat-specific distributions may be found at fairly large scales (e.g., riffles, pools, runs, woody snags, backwaters; see Chapter 2) or at very small scales of resolution (e.g., bottoms compared to tops of stones, along points of laminar flow, see Chapter 4). This basic exercise is designed to introduce the concepts of abundance and diversity within the stream macroinvertebrate community and how these features may differ among habitats within a single stream reach. You will be using the methods described above in *Field Sampling* to obtain quantitative samples from a variety of readily identifiable stream habitats.

15.3.1.1 Laboratory Preparation

1. One set of field-collecting gear (listed below) should be available for every two field-researchers working together as a team.
2. Select an appropriate stream segment from the study stream. This may be based on knowledge that you have acquired from other field work or specifically from work associated with earlier chapters in this book.

15.3.1.2 Field Collection

1. At streamside, identify several different habitat types along a stream length approximately equal to 10 times the width of the stream. Within this segment, you should be able to discriminate among several different habitats.
2. Sketch a simple diagram of the stream reach that you are going to sample (see Chapter 4 for an example of method and detail; also see Chapter 5).
3. If this site has been used for other exercises, refer to your notes regarding patterns of current velocity, substratum size, channel cross-section, spatially explicit habitats, and large woody debris.
4. Delineate and note the range of microhabitats present.
5. Enter the stream and carefully look for macroinvertebrates on cobble, rock outcrops, large wood debris, or other hard surfaces. Make notes concerning your observations.
6. *Special Note:* Some species of macroinvertebrates have very narrow microhabitat requirements and/or may achieve very high densities when environmental conditions are favorable. For example, look closely for black fly larvae (Diptera: Simuliidae). Black fly larvae generally have well-developed “fans” on the head used for straining food

particles from the stream current (Parkes et al., 2004). Because they have narrow flow requirements, black flies often occur in very high abundance in very specialized microhabitats that have a stable substratum and smooth-laminar flows (Malmqvist et al., 2001). Moreover, black fly larvae may exclude other larvae from areas around them by nipping and biting; this territorial behavior often results in uniform spatial distribution patterns. Look for these and other macroinvertebrates that may occur in easily observable areas and note similarities and dissimilarities of microhabitat.

7. Take a sample from each of the different habitat types that you identified. A Surber, Hess, Hauer-Stanford kicknet, or D-frame sampler may be appropriate depending on the type(s) of habitats or sizes of substrata that are present.³
8. Empty the contents of the sampler into a 20-L plastic bucket. Examine each sample for the presence of macroinvertebrates while the sample is in the bucket. At this juncture, you must decide whether samples will be returned to the laboratory for detailed examination or sorted in the field. If the samples are to be preserved immediately and returned to the laboratory for further analysis, go to Step 9. If samples are to be processed in the field, go to the next section on field-sorting.
9. Pour the sample contents from the 20-L bucket into a sieve and let the water drain from the sample. Transfer the sample into an appropriate sized container (e.g., 500-mL jar or 1 qt ziplock bag), place a label containing date, site, and sample number in to the container (use a small piece of paper and pencil), and preserve with 95% ethanol to cover the sample completely and to reach a final concentration of 70% ethanol.⁴

15.3.1.3 Field Sorting and Identification

1. If samples are to be sorted and identified at streamside, pour the contents of the sample from the bucket into an appropriate meshed sieve (e.g., 125, 250, or 500 µm). Refloat the sample by immersing the sieve in water (filling the enamel pan used in the next step with stream water, being careful to not let extraneous organisms into the sample, is a convenient way to do this), being careful not to allow the water to breach the upper lip of the sieve and thereby lose sample contents. Distribute the sample evenly on the sieve-screen and remove the sieve from the water.
2. Using a spoon or butter knife, divide the sample into approximately four equal sections on the surface of the sieve-screen. You have now divided your sample into 1/4 subsamples. Remove one of the 1/4 subsamples, place it into a white enamel pan, add stream water, and distribute the sample around the pan. You should be able to observe many macroinvertebrates crawling or swimming about the pan.
3. It is important to have a sufficient sample size (i.e., one consisting of several hundred individuals). If there are tens of individuals in the pan, then add additional 1/4 subsamples from the sieve, as needed. If there are thousands of individuals, then you will need to further subsample by taking the 3/4 sample remaining on the sieve, returning it to the 20-L bucket and placing the current contents of the 1/4 subsample in the pan back into the sieve. Now go back to Step 1 and reconduct the subsampling procedure. Remember that now the subsamples are 1/16 of the original sample.
4. While invertebrates are still in the enamel pan, examine the body shape of the benthic animals you see from the different microhabitats.
5. Using forceps, remove all macroinvertebrates from the sample (or subsample) and sort them into easily recognized groups. At a minimum, you should be able to identify taxa to the phylum- and order-levels using the general key provided in [Appendix 15.1](#).
6. Place each taxon in a different container.⁵ Count and record the number of individuals within each taxon.
7. For detailed identification and enumeration, the sorted sample must be returned to the laboratory and examined using a binocular dissecting microscope. Place each sorted taxon into a separate container (e.g., scintillation or other glass vial), record the date, site, and sample number on a label for each container using a piece of paper and pencil (do not use a pen; most inks will fade to illegible in ethanol), and preserve with 70% ethanol.

15.3.1.4 Laboratory Sorting, Identification, and Enumeration

1. If samples were not field-sorted, use the laboratory sink and empty the contents of a sample into an appropriate meshed sieve (e.g., 250 or 500 µm). Rinse the sample thoroughly with tap water being careful not to lose any material. Go through the subsample and sorting procedures described in Steps 1–5 above in *Field Sorting and Identification*. Be sure to dilute the used ethanol by at least 10× as you dispose of it in the sink.

3. Note: Collection, sorting and identification of organisms may take several hours per sample; thus, if this exercise is being used within a class setting, we recommend a careful examination of time allocation.

4. Note: Add a small amount of rose bengal to the sample to stain invertebrates and aid in the separation of organisms from debris.

5. Note: Wells of a muffin tin, plastic ice-cube tray, or styrofoam egg carton work well for this purpose.

2. Use the key provided ([Appendix 15.1](#)) to identify the most commonly occurring taxa to the family level for insects and class level for non-insects. If the organism you are identifying does not “key out” or you desire greater resolution in identifications you can use keys in [McCafferty \(1981\)](#), [Pennak \(1989\)](#), [Thorp and Covich \(2014\)](#), [Merritt et al. \(2008a\)](#), and a variety of other sources to separate the various taxa. A binocular dissecting microscope will be needed to view the morphological structures that are used to identify the organisms.
3. *Special Note:* It will not be possible for the beginning student to identify in a single laboratory exercise all the various organisms that typically are found in an unpolluted stream. It generally takes months of work to develop the skills to identify organisms to the generic level and a lifetime of work to the species level.
4. Observe the diversity of species within these taxonomic groups.
5. Select one or two taxonomic groups to examine in detail. For example, you may select to look at the net-spinning caddisflies (superfamily Hydropsychoidea, especially the family Hydropsychidae) or the predaceous stoneflies in the family Perlidae.
6. Carefully sort and identify the individuals from the taxa that you have chosen to study.
7. List the genera/species collected from each of the different habitats. If identification beyond the taxonomic level presented in [Appendix 15.1](#) is beyond your current expertise, then genera/species found within your chosen taxon may be further separated as species A, B, C, etc.

15.3.1.5 Data Analyses

1. Enumerate the selected taxa from each sample collected.
2. Calculate mean density and standard deviation for each selected taxon by habitat.
3. These data for the macroinvertebrate assemblages (e.g., abundance, taxa richness) can be used to calculate various population descriptors.
4. Calculate two common diversity indices that are relative measures of species richness and equitability—the Shannon–Wiener index and Simpson’s index. The Shannon–Wiener information theory index (H') is calculated as

$$H' = - \sum \rho_i \log \rho_i \quad (15.1)$$

where ρ_i , proportion of the total number of individuals in the i th species.

Simpson’s index (λ) is the probability that any two individuals picked at random will be of the same species and is calculated as

$$\lambda = \sum \rho_i^2 \quad (15.2)$$

where ρ_i is as above.

Simpson’s index is a measure of the extent that individuals in a sample are concentrated into a few species. See Chapter 38 for biotic indices using stream macroinvertebrates.

Special Note: Sorting, identification, and enumeration of macroinvertebrate samples can be very time-consuming for even the most accomplished aquatic entomologist or benthic ecologist. ([Merritt et al., 2008a](#)) provides an excellent flow diagram summarizing the general procedures for analyzing benthic samples. Depending on taxonomic complexity, abundance, and extent of analyses (e.g., enumeration, wet weights, ash-free dry mass) a single sample may take 8–10 h (or more) spread over several days to completely sort and identify.

15.3.2 Basic Method 2: Watershed Scale Distribution

Stream ecologists have noted that particular macroinvertebrate species often occur only within very restricted stream reaches. In some cases, this is because the habitats that particular species require only occur within certain well-defined reaches. Although various habitat types occur along the entire length of the stream (e.g., riffles), one will find many species that are reach-specific along the river continuum. This exercise is designed to illustrate the macroinvertebrate species-replacement that can occur along the downstream gradient of a river network. In this exercise, we will collect macroinvertebrates from riffles, runs, backwaters, and other habitats that can be differentiated following the protocols outlined in Chapters 2, 4, and 5. Depending on your specific research question, you may examine the macroinvertebrate assemblages from each stream-reach looking for changes in species composition with an emphasis on closely related species, or you may decide to distribute your effort across the entire macroinvertebrate assemblage. In the event that this is an exercise to be accomplished by a class or special research project, we suggest that you focus your attention on the net-spinning

caddisflies (Trichoptera: Hydropsychoidea) for this exercise, because they occur in almost all unpolluted running water systems, especially in riffles of gravel-bed streams or on stable substrata (e.g., woody snags) in sandy bottom streams or rivers. Also, the net-spinning caddisflies are easily recognized and larval taxonomy is fairly well known for many areas of North America and Europe.

15.3.2.1 Laboratory Preparation

1. Select a fourth- to fifth-order stream network using a detailed watershed map(s) (e.g., USGS quadrangle map, scale 1:24,000).
2. Select a series of sampling sites along the stream-river longitudinal corridor, with consideration for ease of access and diversity of habitats among and within sites. A site should be chosen to represent each order of stream. The researcher should be well versed prior to going to the field, carefully considering the number of replicate samples to be taken within each of the habitat types identified following a habitat mapping exercise at each site (see Chapters 4 and 5).
3. Consider the various physical and biological variables that may affect macroinvertebrate distribution and abundance. Determine which of these factors are to be investigated along with the collection of the benthic samples. Following the protocols for mapping (Chapters 4 and 5), collecting velocity data (Chapters 3 and 5), temperature (Chapter 6), groundwater/surface water interactions (Chapter 8), or other physical and biological variables, lay out a strategic plan for gathering and recording the data.

15.3.2.2 Field Collection

1. Obtain quantitative samples from each habitat using the technique described above in *Field Sampling* of Basic Method 1 (e.g., a Hauer-Stanford kicknet, Surber, or Hess sampler) for riffle habitats and dip-net samplers for backwater areas where the current is not sufficient to transport dislodged macroinvertebrates into the net.
2. If this is a rapid examination exercise, examine each sample for the presence of the macroinvertebrate guild of interest (e.g., hydropsychid caddisflies, perlid stoneflies, or ephemerellid mayflies). Place contents of the sample into a white, enamel pan and use forceps to remove the specific taxa of interest from the sample material. Place larvae into a suitably sized plastic jar with 70% ethanol, using a single jar for each sample. The remainder of the live sample may be preserved for additional examination or returned to the stream.
3. If this is a comprehensive study, collect all organisms, place them into a sieve or small net to remove excess water, and place the contents of the sample into a plastic jar with 70% ethanol, again using a single jar for each sample. Be certain to label the samples by placing a label affixed to the outside of the jar and on a piece of paper inside the jar.
4. Collect all relevant biological and physical data for each sample collected (e.g., riparian vegetation, substratum characteristics, current velocity, depth, etc.).

15.3.2.3 Laboratory Analysis

1. After bringing samples back to the laboratory, identify target taxa to the genus- or species-level. Use a binocular dissecting scope to examine the organisms and their key morphological structures. Merritt et al. (2008a) provide generic-level keys; however, various regional keys for identification of species are available. (For illustrative purposes, you can use “morphospecies”; e.g., Species A, B, C, etc.)
2. Observe the morphological diversity and taxa richness of species within the taxonomic and functional group(s) with which you are working.
3. Carefully sort, identify, and enumerate the individuals of each taxon from each sample.
4. Calculate the abundance for each species by sample and determine means, standard deviations, and Shannon–Wiener and Simpson’s indices by site and by habitat.

15.3.3 Advanced Method 1: Population Dynamics and Movement

Populations change in size over time, increasing from new births and the immigration of individuals from other areas, and decreasing from death and emigration. In this exercise, we will mark members of populations of aquatic insects to observe their movements over time, as well as losses (emigration, death) or gains (immigration, births) to the population. (See also Chapter 20 for a more detailed discussion of marking stream invertebrates).

Water striders (Family Gerridae, and the species you likely have, is *Aquarius (=Gerris) remigis*) *A. remigis* is one of the most widely distributed species of aquatic insect, and it occurs on several continents. Individuals commonly occur in the slow-flowing margins and pools of streams [see [Spence and Anderson \(1994\)](#) for a detailed review of the biology of water striders]. Using a handnet, catch one of these surface-dwelling creatures. Note that its “back” (i.e., the dorsum of the thorax) is where we will apply our marking tag—a dab of typewriter correction fluid (“white-out,” which comes in a variety of colors and by using up to three marks on an insect and different colors, scores of individuals can be marked and followed).

15.3.3.1 Behavioral Observations

1. Working in pairs, you will describe the spatial distribution of *A. remigis* (or some other insect such as a cased limnephilid caddisfly such as *Dicosmoecus*; see below).
2. Map a segment of stream (~50-m reach) following the examples in Chapters 2, 4, and/or 5 (or if you are working in the same location, use the maps created in an earlier exercise).
3. Collect and sex (in the case of water striders) each individual (seeing the two genders side-by-side makes this pretty clear; the female is larger); then mark them by using a code based on a series of different colors, and release them where you caught them.
4. Observe the behavior of individual water striders with respect to their location in the stream, and their resting, mating, searching, fighting, and feeding behaviors. Watch each individual for 10–15 min, and be sure to compare individuals of different sex and maturity.
5. Make detailed notes on each of these behaviors and the time they spent doing each activity.

15.3.3.2 Mark and Recapture

1. A final exercise is a mark-recapture study (see Chapter 16 for detailed rationale and assumptions underlying mark-recapture studies).
2. This method involves sampling on two days, about one week apart.
3. Record the number of individuals originally marked on day 1, the number collected on day 2 that were marked and unmarked, and then calculate population size (N) as follows:

$$N = \frac{M \times C}{R} \quad (15.3)$$

where M , number originally marked; C , total catch on day 2; and R , the number of day 2 recaptures (i.e., those originally marked on day 1).

4. *Special Note:* The larger cased caddisflies in the family Limnephilidae (e.g., *Dicosmoecus* spp.) are also appropriate for this type of study (see [Appendix 15.1](#)). To mark each individual, remove the larva and case from the water, pat the case dry, add the mark (use colored, permanent-marker pens), and then return the caddisfly to the stream at the place collected. Another interesting exercise is to compare upstream/downstream movements of marked larvae that have all been released at a single point.

15.3.4 Advanced Method 2: Laboratory Artificial Stream Experiments

Many experiments can be conducted in laboratory streams with aquatic macroinvertebrates (see [Hauer, 1993](#)). [Lamberti and Steinman \(1993\)](#) provide many designs and applications of laboratory streams. In this exercise, we will construct several small airlift chambers that provide the microhabitat-flow requirements needed by black flies (Simuliidae) and determine larval growth rates under different environmental conditions. See [Hauer and Benke \(1987\)](#) for detailed methods and the construction and operation of these small artificial stream tanks.

15.3.4.1 Setup and Experimentation

1. Construct at least four artificial stream tanks.
2. Using tropical fish aquarium supplies (listed below) arrange the artificial stream tanks to provide an “airlift” current when the air pump is on (see [Hauer and Benke, 1987](#)).

3. Water level in the artificial stream tanks should be maintained as nearly full, but not so full that water spills over the top.
4. Obtain black fly larvae from a nearby stream (typically found in shallow, high-velocity habitats) and return live specimens to the laboratory in a large bucket. Collect at least 200–250 individuals in midsize classes (3–4 mm).
5. Remove a random sample of 15–20 animals of the population and preserve in 70% ethanol.
6. Distribute the remaining animals randomly among the four artificial stream tanks. Maintain tanks and permit larvae to feed and grow over a 1- to 2-week period. You will need to add replacement stream water to the tanks twice daily throughout the experiments to maintain water levels and natural food levels. This is best done by bringing unfiltered stream water to the laboratory in 20-L carboy containers. Keep track of the amount of water added over the duration of the experiments.
7. Experimental conditions may be varied among the artificial stream tanks. For example, some tanks may be kept at cool temperatures in a refrigerator or environmental chamber while other tanks are maintained at room temperature. Likewise, some tanks may be given a supplemental food source of either cultured algae, natural seston collected from a stream, or small quantities of granular baker's yeast.

15.3.4.2 Analysis of Growth Experiments

1. Terminate the growth experiments after 10–14 days or as soon as the first individuals begin to pupate, whichever comes first.
2. Keeping larvae from each tank separate, collect and preserve animals in 70% ethanol.
3. Using a dissecting microscope fitted with an ocular micrometer, measure the total length of all larvae from each experimental stream tank and the larvae that were preserved at the start of the experiments.
4. Dry mass (DM) of each larva may be predicted from the regression (Hauer and Benke, 1987)

$$DM = 0.0031 \times BL^{2.64} \quad (15.4)$$

where BL, body length in mm.

5. Calculate daily instantaneous growth rates (g) for larvae as

$$g = \frac{\ln(DM_f/DM_i)}{t} \quad (15.5)$$

where DM_f , mean dry mass (in mg) of larvae at the end of the growth experiment; DM_i , mean dry mass of the larvae at the start of the experiment; and t , number of days for the particular trial.

15.4 QUESTIONS

1. Did you observe specific macrohabitat preferences (e.g., riffle, pool, backwater)? Did you observe specific microhabitat preferences (e.g., top, side, or bottom of rock within a riffle)? How do these relate patterns to the morphological and behavioral adaptations described in the Introduction?
2. Were you able to see morphological differences among the species that you collected from different habitats? How did the morphology of species collected from riffles differ from species collected from pools, debris dams, or leaf litter?
3. Consider the breadth of different habitats that you have observed in stream ecosystems. Imagine that you are standing next to a stream whose bottom and sides are concrete. In your mind or on paper consider how you would remake this concrete channel into a living stream. What structural components would you add to increase microhabitat complexity (and hence abundance of organisms)? Consider the stream bed. How would you integrate the hyporheic zone into your imagined stream? Where do factors such as riparian vegetation and nutrient sources come into play? You've now begun to think about stream restoration.
4. Did you observe distinctly different species within the larger taxonomic group that you identified? Even though you may not have been able to identify your specimens to the species level, how many different putative taxa were you able to distinguish?
5. Did you observe a pattern of different species among sample sites along the longitudinal gradient of the stream within the taxonomic group that you studied in detail? What general patterns of species distributions or replacements did you observe?
6. How do water striders respond to differences in flow? On your original map, record areas of fast, medium, and slow flow (see Chapter 4) and compare to strider distribution. Did sex, age, or other factors within the water strider

experiments appear to influence distribution? Can food resources you provide be used to alter microhabitat selection? What are some assumptions that we make about the effect of the mark on the animal when we conduct such experiments?

7. If you conducted growth experiments, what was the growth rate of black fly larvae from each of the experimental stream tanks? Did different temperatures or different levels of food resources affect growth rate?
8. Consider instantaneous growth (g). What relationship does g have to secondary production? (see Chapter 35).

15.5 MATERIALS AND SUPPLIES

Field Materials

- 20L plastic bucket(s)
- $\leq 500\text{-}\mu\text{m}$ sieve or small bag-net of this mesh
- 95% ethanol
- Current velocity meter
- D-frame net, Surber sampler, Hess sampler, or Hauer-Stanford kicknet
- Meter sticks
- Permanent marker pens (variety of colors)
- Stop watch
- Typewriter correction fluid (various colors; for Advanced Method 1)

Laboratory Equipment and Materials

- 70% ethanol
- [Appendix 15.1](#) and reference books mentioned in text
- Artificial stream tank, air pump, tubing (for Advanced Method 2)
- Binocular dissecting microscope
- Forceps
- Scintillation vials or patent-lip vials with neoprene stoppers
- White enamel pans for sorting

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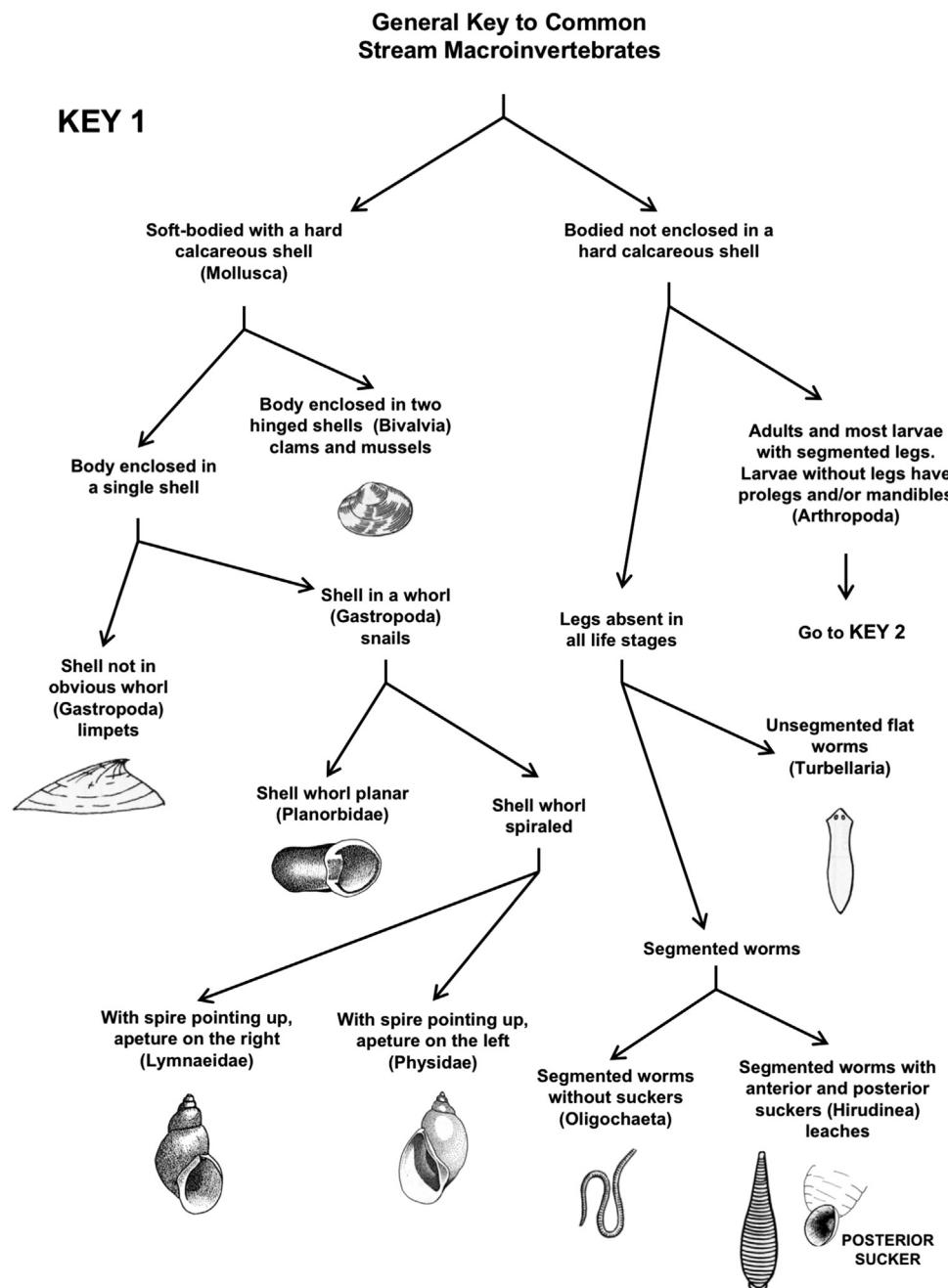
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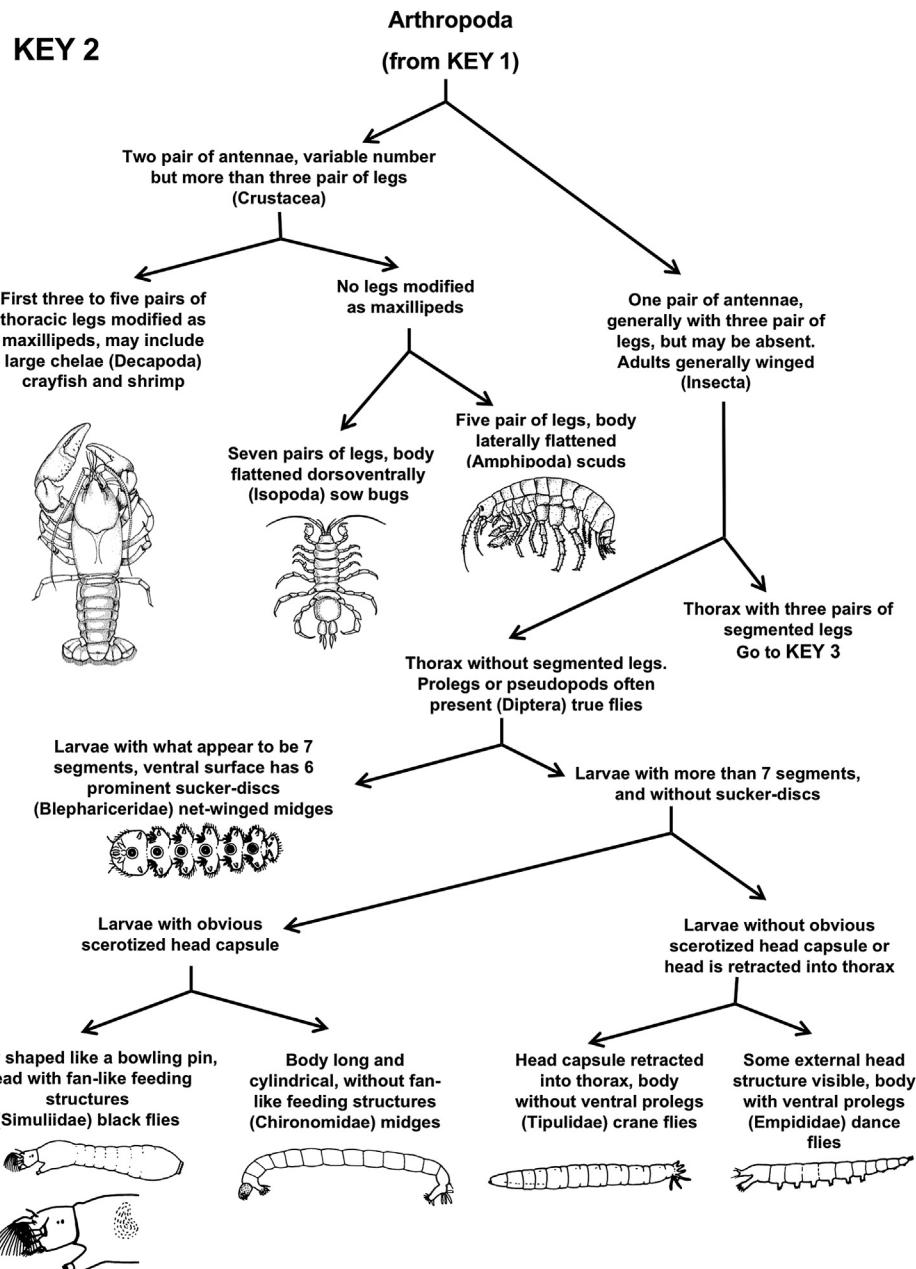
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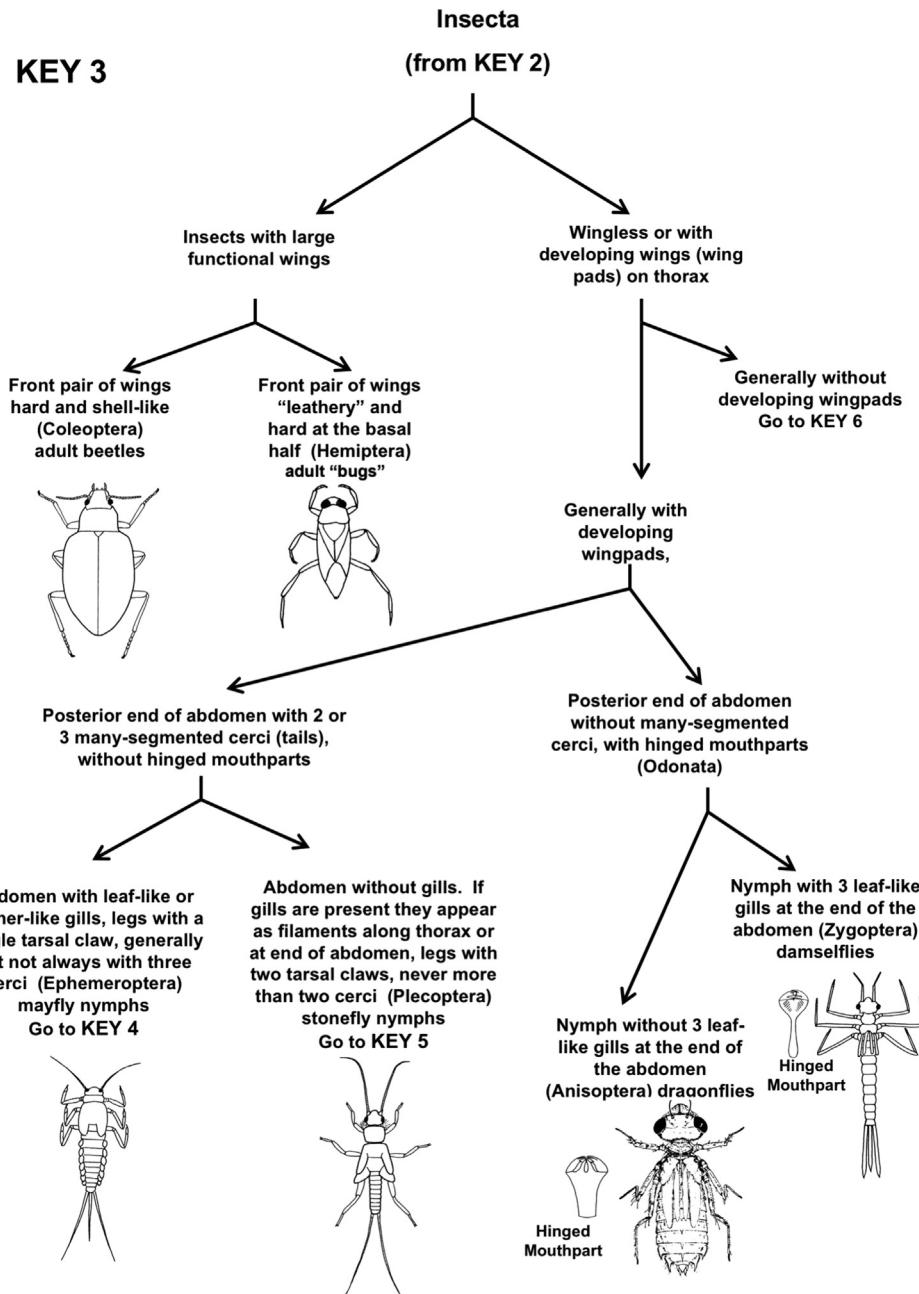
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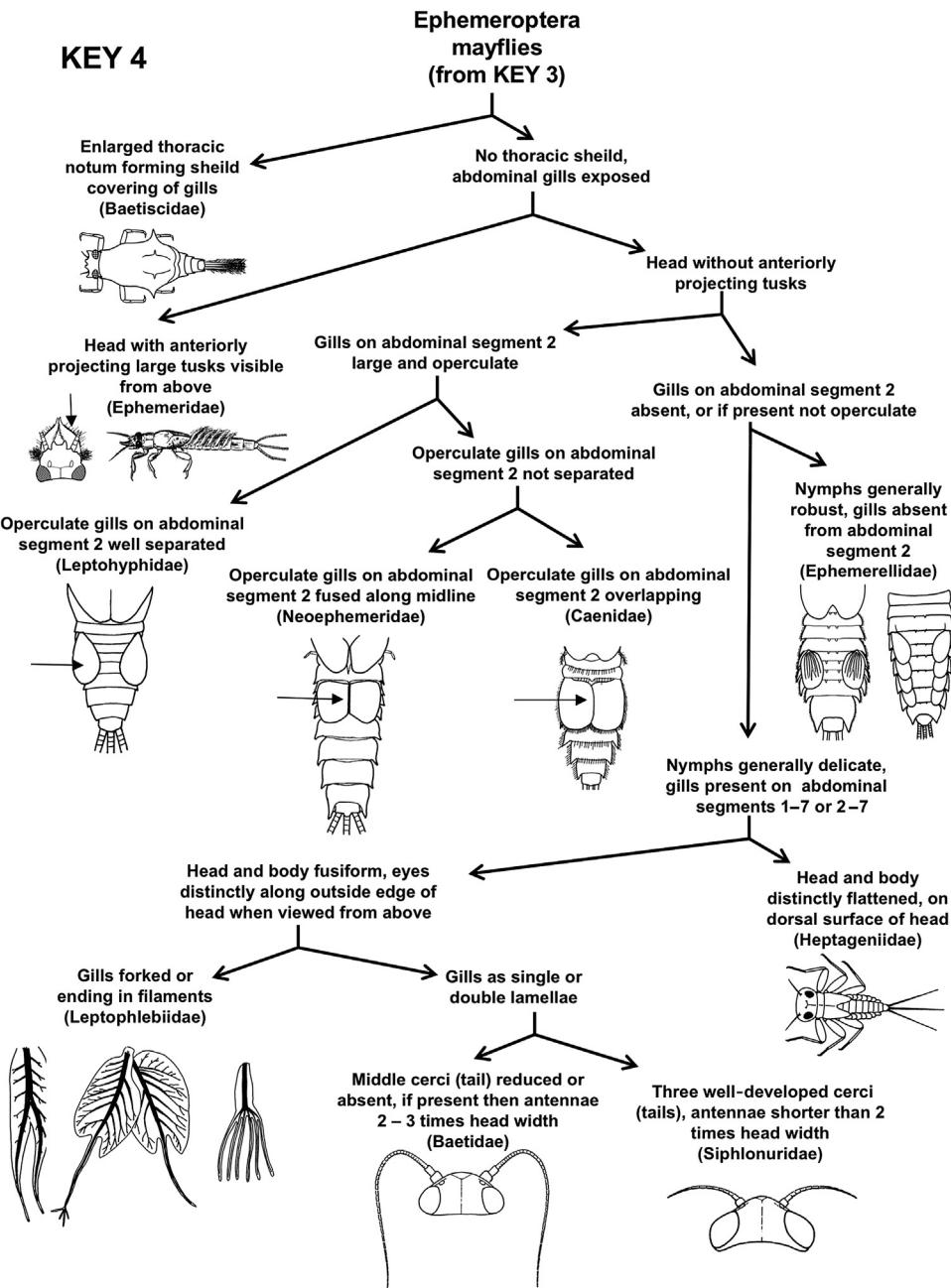
APPENDIX 15.1

A simplified key for the rapid identification of the most common stream macroinvertebrates. Non-insect taxa are described to the phylum or order level. Insect taxa are described to the family level. Many more stream macroinvertebrates occur than are presented here; this key is intended to only serve as a starting point for their identification. (Some illustrations taken from Betten, 1934; McCafferty, 1981; Thorp and Covich, 2001, with permission).



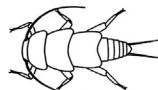






KEY 5**Plecoptera
stoneflies
(from KEY 3)**

Nymph "roach-like",
thoracic sterna appear as
large plates

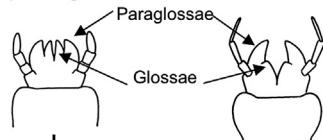


Nymph not "roach-like",
thorax not as large plates

Branching gills at the
base of the legs

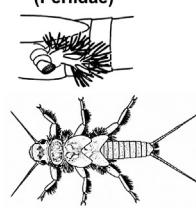
No branching gills at
the base of the legs

Mouth-parts with 3
distinct notches formed
by the glossae and
paraglossae being of
equal length



Mouth-parts with a single
wide notch formed by the
paraglossae positioned
well forward of the glossae

Branching gills only at
the base of the legs, never on
abdominal segments 1 or 2,
gills may be present at
posterior of abdomen
(Perlidae)



Branching gills at the base
of the legs, and abdominal
segments 1 and 2
(Pteronarcyidae)



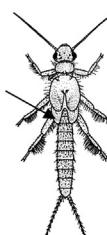
Thorax robust, with
divergent wingpads



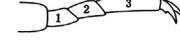
Thorax slender, with
parallel wingpads



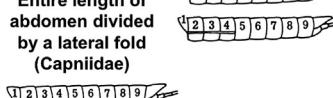
Wingpads parallel
forming a distinct U,
cerci (tails) shorter
than abdomen
(Chloroperlidae)



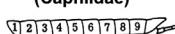
Tarsal segments 1 and 2
about the same length
(Taeniopterygidae)



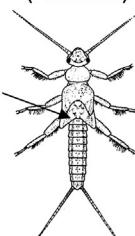
Lateral fold along
abdomen only
extending about half
way and no farther
than segment 7
(Leuctridae)



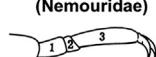
Entire length of
abdomen divided
by a lateral fold
(Capniidae)

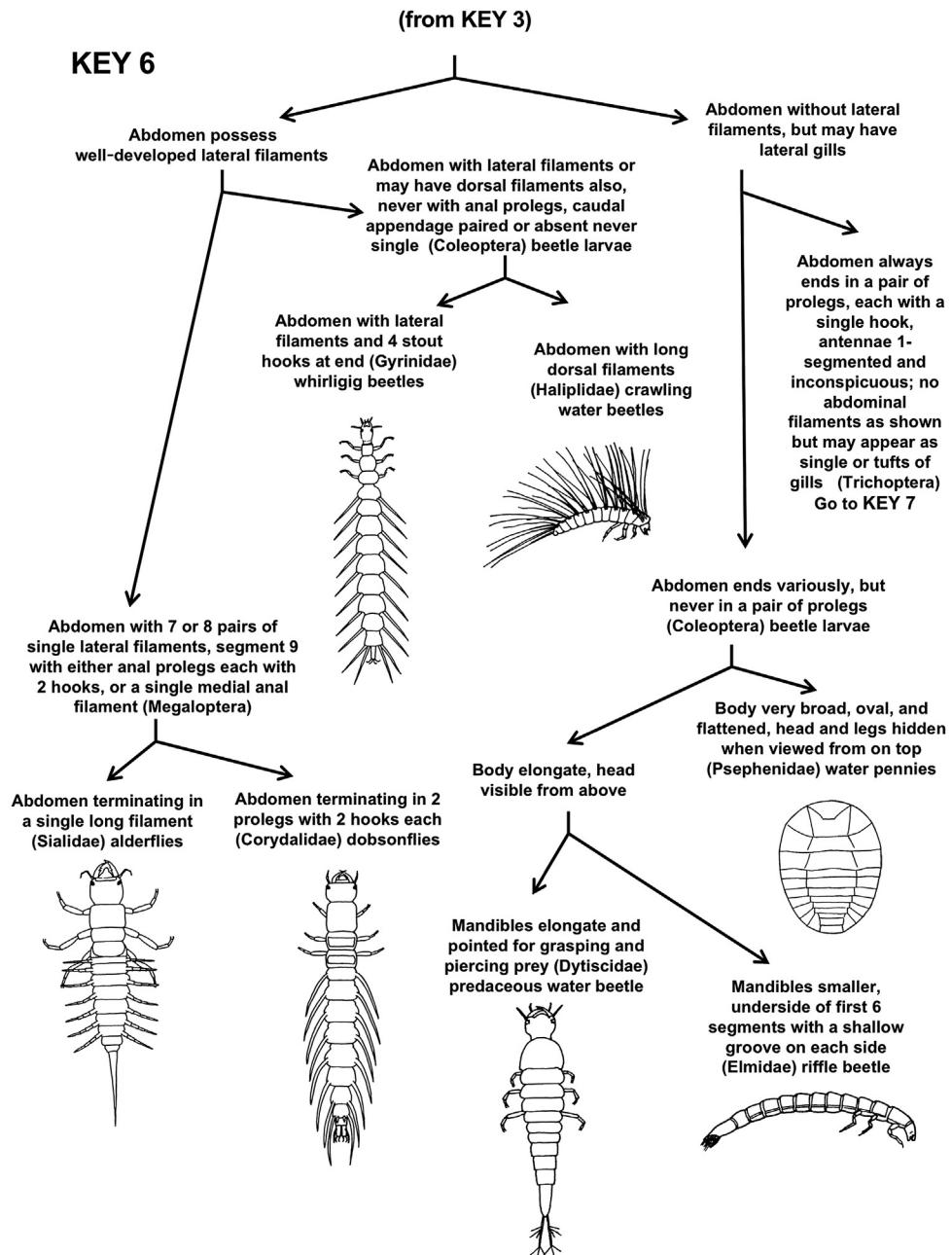


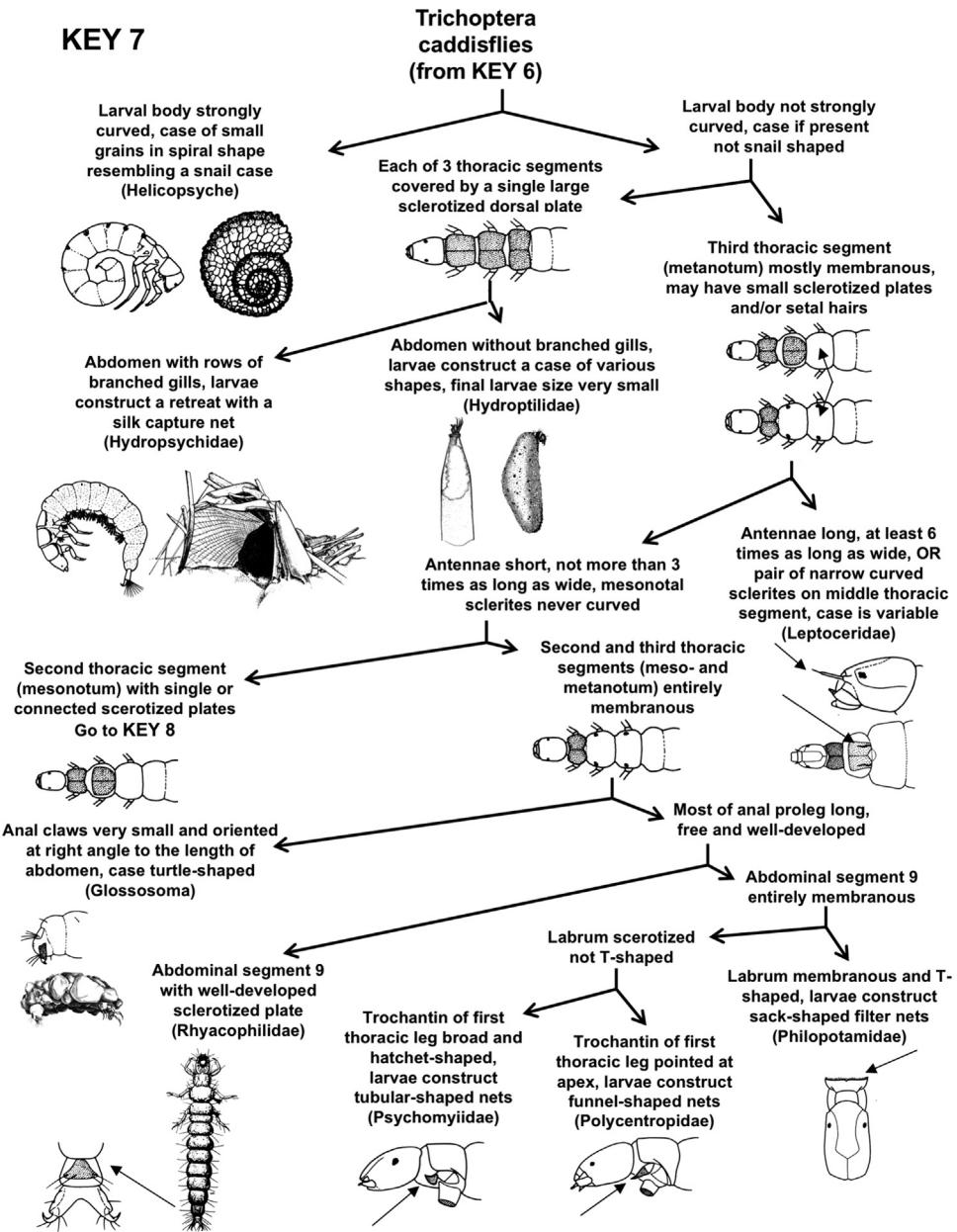
Wingpads divergent
forming a distinct V,
cerci (tails) as long or
longer than abdomen
(Perlodidae)



Tarsal segment 2 much
shorter than segment 1
(Nemouridae)

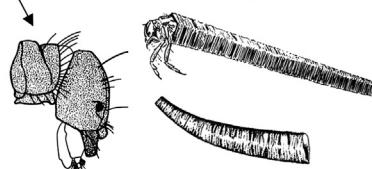






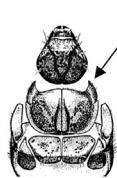
KEY 8**Trichoptera
caddisflies (cont.)
(from KEY 7)**

Pronotum divided by a deep furrow, no dorsal or lateral humps on abdominal segment 1; case often square in x-section but may be round (Brachycentridae)



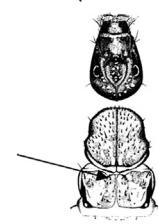
Antennae located about midway between eye and mandibles; median dorsal hump present on abdominal segment 1

Mesepisternum formed anteriorly into a sharp elongate process (Goeridae)

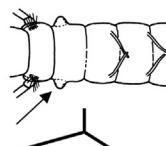


Mesepisternum not formed into a sharp elongate process

Notch along the mesal suture (midpoint along anterior edge) of the mesonotum (Unionidae)



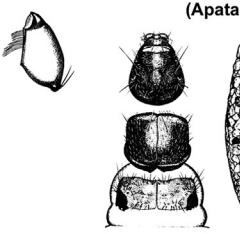
Pronotum not divided by furrow, first abdominal segment with lateral hump and usually also dorsal hump



Antennae located extremely close to the eye; median dorsal hump absent; case variable of wood or stones (Lepidostomatidae)



Mandibles with uniform scraping blades; case usually cornucopia-shaped (Apataniidae)



Mandibles not modified into scraping blades; cases are highly variable of plant and mineral materials and in many shapes; many of the larvae in final instar prior to pupation are large (>12mm) (Limnephilidae)

