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Chapter 14

Meiofauna

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14.1 INTRODUCTION

The meiofauna (or meiobenthos) are the smallest metazoans dwelling in the substratum of streams and other stationary surfaces, such as tree root wads and other large debris. They are roughly defined as metazoans that can pass through a 500-μm sieve, but are retained on a 40-μm sieve (Higgins and Thiel, 1988). Meiofauna are diverse, numerically dominant, and act as trophic intermediaries between micro- and macroscopic organisms in stream ecosystems (Schmid et al., 2000; Schmid-Araya et al., 2002a). In streams, the ecological role and importance of the benthic meiofauna might be similar to that of the zooplankton for lakes and large rivers. Meiofauna can be distinguished as permanent (fully benthic species that remain in the meiofaunal size-range during their whole lifespan), or temporary (species that start off as meiofauna but grow into macrofauna, or emerge out of benthos during their lifespan). Nematodes, rotifers, and harpacticoid copepods often dominate permanent meiofaunal communities, although curious animals such as tardigrades (also coined "water bears"), ostracods, cladocers, gastrotrichs and microturbellarians can be found in some habitats (Fig. 14.1). Temporary meiofauna are typically dominated by the youngest instars of aquatic insects (especially chironomids), but also comprise oligochaetes and water mites.

Meiofauna are fascinating organisms that have attracted the attention of zoologists since the earliest development of microscopy (i.e., the "animalcules" first observed by Van Leeuwenhoeck in 1677). Meiofaunal communities commonly exceed millions of individuals from hundreds of different species coexisting within only one square meter of a riverbed (Robertson et al., 2000). Meiofauna can be found worldwide, from glacier-fed rivers to thermal springs, from oligotrophic to eutrophicated waters, and they massively inhabit groundwater biotopes (e.g., Ward and Palmer, 1994; Rundle et al., 2000; Traunspurger, 2000; Eisendle-Flöckner and Hilberg, 2014). Epibenthic meiofauna colonize microbial mats (biofilms) coating bedrock, boulders, pebbles, macrophytes, mosses, riparian tree roots, fallen branches, and leaf litter packs (Fig. 14.2). These typically are the largest members of the meiofauna and often are good swimmers or secrete adhesive substances to resist being transported by downstream flow. In contrast, interstitial meiofauna colonize streambed sediment particles and typically are small, worm-shaped, and poor swimmers (Fig. 14.3). Many interstitial species also have adhesive organs for attaching to sand grains. Burrowing meiofauna live in fine sediments and often have robust bodies for pushing aside mud and silt.

Meiofauna are disseminated by surface and subsurface flow, biofilm detachment and drift, wind, rain run-off, and traveling animals (Palmer, 1992; Ward and Palmer, 1994; Gaudes et al., 2006; Frisch et al., 2007; Ptatscheck and Traunspurger, 2015). In addition, some species can enter tough dormant stages—dormant tardigrades can even "revive" after a few days of exposure to outer space (Jönsson et al., 2008). Meiofauna communities are extremely resilient to the heaviest of disturbances, like severe droughts and spates. Species distributions tend to be patchy and stochastic, species assemblages are mostly shaped by specific adaptations to habitat characteristics and by interactions with other organisms (Robertson, 2000; Silver et al., 2000; Swan and Palmer, 2000; Gaudes et al., 2010; Majdi et al., 2012a; Tahseen, 2012). Despite important taxonomical works, many species are still new to science, suggesting that the contribution of meiofauna to the diversity of life in streams is currently underestimated. Meiofauna deserve considerable additional work—as exemplified by the recent discovery in polar cold springs of a species, *Limnognathia maerski*, which is the unique representative of an entirely new phylum: the Micrognathozoa (Kristensen and Funch, 2000).

Dissolved oxygen, organic matter content, and water flow may be the most important abiotic factors regulating meiofaunal populations at small scales in streams and rivers (Swan and Palmer, 2000; Beier and Traunspurger, 2003; Caramujo et al., 2008;

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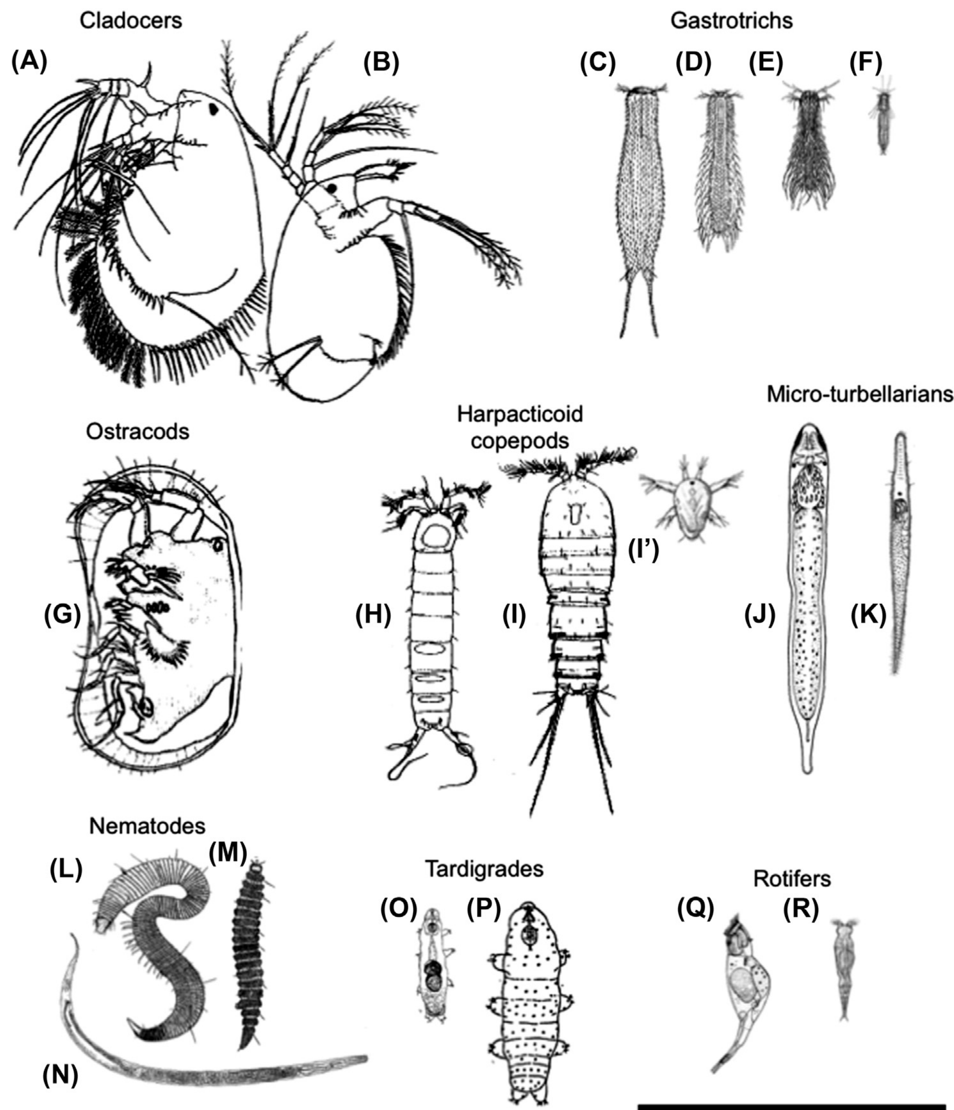

FIGURE 14.1 Some examples of permanent freshwater meiofauna taxa morphotypes: (A) Ilyocryptidae. (B) Macrothricidae. (C) *Polymerurus*. (D) *Euchaetonotus.* (E) *Hystricochaetonotus.* (F) *Proichthydioides*. (G) Limnocytheridae. (H) *Parastenocaris*. (I) *Bryocamptus* and (I') Nauplius larvae. (J) *Stenostomum.* (K) *Rhynchoscolex*. (L) Epsilonematidae. (M) *Desmoscolex*. (N) *Monhystrella*. (O) *Hypsibius*. (P) *Macrobiotus*. (Q) *Proales*. (R) *Philodina*. Specimen are at same scale (bar: 1 mm). *Modified from Cuénot (1932), Kolasa et al. (1987), Rundle et al. (2002), Noreña et al. (2005), Abebe et al. (2006), and Giere (2009) after various sources.*

Majdi et al., 2011, 2015a). Most stream meiofauna are obligate aerobes, and several studies have found a correlation between oxygen and meiofaunal populations (e.g., Boulton et al., 1991; Beier and Traunspurger, 2003). Because dissolved oxygen and interstitial water flow are influenced by sediment grain size and depth, these latter variables should be good predictors of meiofaunal abundance and composition in many cases (Ward and Voelz, 1990; Caramujo et al., 2008; Reiss and Schmid-Araya, 2008; Rae, 2013). For instance, gravel harbors an abundant and diverse meiofauna, particularly rotifers, copepods, and tardigrades, whereas sands and silts are inhabited chiefly by worm-shaped oligochaetes, chironomids, and nematodes. Other physicochemical factors (e.g., temperature and pH) may also be important regulators at regional scales (Rundle and Ramsay, 1997; Zullini et al., 2011).

Some meiofauna are sensitive to pollutants, and should be useful as indicator species (see Section 14.3.4) because they are "frontline" organisms, facing sediment pollution during their whole life cycle and for several generations (see reviews of Traunspurger and Drews, 1996; Bongers and Ferris, 1999). As an example, assemblages of freshwater nematode species have been highlighted as subtle indicators of sediment contamination by heavy metals and xenobiotics (Heininger et al., 2007; reviewed in Hägerbäumer et al., 2015). A trait-based index has even been validated to link specific environmental stress to the structure of nematode communities: the "nematode species at risk" index (nemaSPEAR; Höss et al., 2011). On the other hand, meiofauna also emerge as promising auxiliaries for dealing with organic pollution. For instance, some

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FIGURE 14.2 Illustration of an epibenthic biofilm community with colonial and stalked diatoms and Chromadorid nematodes. *Adapted from Meschkat (1934).*

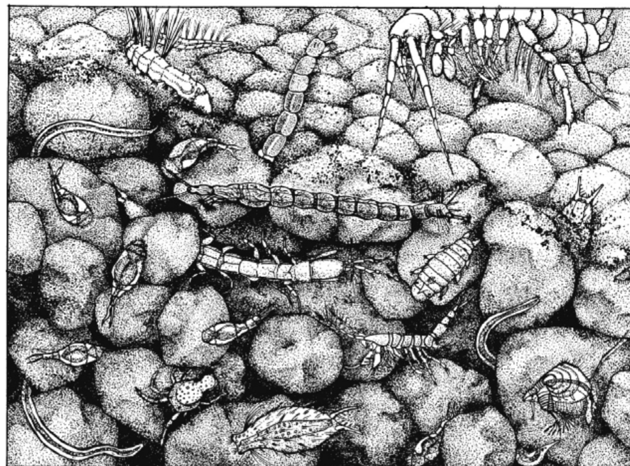

FIGURE 14.3 Illustration of a freshwater interstitial community dominated by meiofauna. *From Giere (2009).*

meiofauna (especially oligochaetes, bdelloid rotifers, and some nematodes) are quite robust and can thrive in organically polluted environments. These meiofauna are known to colonize filters in water treatment plants and wastewater filters (Schreiber et al., 1997; Fried et al., 2000; Bergtold et al., 2007) where they graze and break through biofilms, skimming off bacterial mats. This maintains good filtration rates for membranes used in municipal drinking water (Derlon et al., 2013). Recent evidence suggests that meiofauna's "micro-bioturbation" can even increase the rate at which nitrate is reduced by streambed microbes (Liu et al., 2015, 2016).

Meiofaunae show marked seasonality in reproduction and abundance. In most temperate streams, the density and secondary production of meiofauna populations peak from late spring through early fall (Kolasa et al., 1987; Palmer, 1990; Suren, 1992; Beier and Traunspurger, 2003; Reiss and Schmid-Araya, 2008; Traunspurger et al., 2015; Majdi et al., 2016a). Meiofauna generally represent >95% of the benthic animals in most streams, and are energetically important due to fast growth and turnover rates (see Chapter 35). Information on the functional role of stream meiofauna is still relatively

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sparse, owing to the limited number of studies on this topic (reviewed in Hakenkamp et al., 2002). For example, Tod and Schmid-Araya (2009) estimate a minor contribution of meiofauna to total invertebrate production in a macrophyte-covered streambed. However, Poff et al. (1993), Stead et al. (2005), and Majdi et al. (2016a) support the idea that meiofauna make a significant contribution to invertebrate production and stream metabolism in a variety of habitats.

The activities of stream meiofauna (e.g., locomotion, excretion, grazing, scraping, filtration) affect microbial communities, detrital dynamics, and even fine particle transport (e.g., Perlmutter and Meyer, 1991; Borchardt and Bott, 1995; Traunspurger et al., 1997; Kathol et al., 2011). Interestingly, although meiofauna are predominantly microbivores, their presence seems to stimulate bacteria abundance, as well as the activity of bacteria (Traunspurger et al., 1997) to increase the photosynthetic activity of microalgal biofilms (Mathieu et al., 2007) and to favor nutrient uptake in phototrophic and interstitial biofilms (Liu et al., 2015, 2016). Stream meiofauna are indirectly affected by habitat-engineering predators (Majdi et al., 2014, 2015b, 2016b), and are also a direct food resource for many benthic macroinvertebrates (e.g., Schmid and Schmid-Araya, 1997; Beier et al., 2004; Ptatscheck et al., 2015), and juveniles benthivorous fishes (e.g., Spieth et al., 2011; Weber and Traunspurger, 2015). In short, compelling evidence indicates that meiofauna play a significant ecological role in stream ecosystems by affecting important food web metrics, such as complexity and connectance (Schmid-Araya et al., 2002a, 2002b).

Although our understanding of the contribution of meiofauna to stream ecosystem functioning has grown in the past decade, it still remains insubstantial in comparison to what we know about the contributions of microbes and macrofauna. There is an urgent need to carry out large-scale quantitative evaluations of the interactions of meiofauna with other biota and ecosystem processes, and to embed meiofauna in routine investigations of stream ecosystems (Giere, 2009). Stream ecology has been a fast-growing field of research over the past few decades. To fully grasp the complexity of stream ecosystem structure and functions, meiofauna must be included in the picture. We also believe that meiofaunal communities are relevant indicators for better understanding the consequences of the threats that stream ecosystems increasingly face. In this context, the objectives of this chapter are to provide a general background and a toolbox for freshwater meiobenthologists. First, we provide an overview of field sampling and the basic methods for the observation, identification, and quantification of meiofauna. Second, we provide an overview of advanced methods: (1) to investigate taxonomy and species distribution using molecular tools, (2) to assess the effects of toxicants on surrogate measures of fitness using laboratory assays, and (3) to investigate trophic interactions using a variety of laboratory and field approaches. At the end of the chapter, we provide a list of the materials and supplies that are required for the basic methods; the advanced methods should be undertaken only after consulting the key references we provide.

14.2 GENERAL DESIGN

14.2.1 Site Selection

Studies of meiofauna may be either qualitative or quantitative, depending on the research goals. For qualitative and behavioral work, the new investigator may wish to first simply scoop up fresh stream substrata and observe live meiofauna. To observe the greatest meiofaunal diversity, such a first look should be ideally performed in a relatively pristine stream and at a site with good water flow and medium to coarse substrata. Finer substrata (e.g., mud) do harbor large numbers of animals, but samples must be collected only in the top layer of sediment, and care must be taken not to "poison" the sample with deeper, anoxic mud. Microbial biofilms coating the cobbles, mosses, macrophytes, and fallen branches and leaves also harbor copious amounts of meiofauna, allowing comparison of meiofaunal communities colonizing different habitats. Note that in the warm summer months, animals left unpreserved will decay within hours. Thus, live samples must be quickly stored in a cooler.

For quantitative ecological work, samples are easily collected and preserved using well-defined techniques. For quick identification of major taxa, refer to Fig. 14.1 or use general references such as Higgins and Thiel (1988), Smith (2001), Rundle et al. (2002), Giere (2009), or Thorp and Covich (2009), and for insects use Merritt et al. (2008). If species-level identifications are needed, the bibliographies of these sources should be consulted along with recent taxonomical monographs (e.g., Schmidt-Rhaesa, 2014 for nematodes).

14.2.2 Sampling

14.2.2.1 Qualitative Collection of Live Animals for the Classroom and for Experimentation

Examining the live fauna is quite instructive, and a variety of data should be collected on locomotion, feeding, and sexual behavior. Additionally, for many taxa, species-level identifications cannot easily be made on preserved material (e.g., bdelloid rotifers, microturbellarians, gastrotrichs).

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The easiest way to collect live meiofauna from coarse sediment is the swirl-decant procedure: A shovel of substratum should be removed from the streambed and poured into a 10-L bucket. Add over 5−6 L of filtered stream water, and stir the sediment vigorously to suspend fine particles and animals in the water. After a few seconds of waiting for the heavy particles to settle, pour the supernatant through a sieve. Repeat this procedure five or six times to extract most of the animals from heavier sediment particles. Using a wash bottle, rinse the animals retained on the sieve into sample jars with filtered stream water. The swirl-decant process can be performed in the field or in the laboratory as long as fresh stream water is used. If animals are to be examined later, it is imperative that the samples of fresh material be kept cool. We routinely keep fauna alive for several days if we store the samples in a cool environmental chamber or a refrigerator.

The composition and abundance of the fauna collected depend strongly on the mesh size of the sieve (Hummon, 1981). Using a 125-μm sieve is acceptable for classroom exercises; however, a 40-μm sieve is more appropriate for research settings. Nonetheless, be aware that even a 40-μm mesh size may still result in the loss of a many of the smallest rotifers and nematodes (Hummon, 1981). A 10-μm sieve ensures the recovery of the very smallest meiofauna, but it probably should be stacked with the larger meshes of 125 and 40-μm sieves. Even then, the investigator should gently shake the sieve series throughout the filtering process to reduce clogging. Sieves are economically made from Nitex mesh and large plastic jars or from PVC-plumbing drain pipe. For instance, one can cut off the end of a plastic jar and a large circle out of the jar lid and secure the mesh under the jar lid by screwing the lid on tightly over the mesh. Likewise, thin-walled 8−10 cm diameter PVC drain pipe obtained from a plumbing supply store also works well. If this route is used, purchase several PVC couplers and a length of pipe. Cut the pipe into 12−14 cm lengths. Place the Nitex mesh over one end of the pipe, and slide the coupler over the Nitex and onto the pipe, thereby tightly holding the Nitex mesh in place.

Other methods for collecting live animals from a variety of benthic habitats have been employed. Bou-Rouch pumps are useful in large gravel or cobble streambeds that are difficult to penetrate. The pump stand has a perforated lower hollow column into which water and associated fauna can seep and from which water is then pumped up and sieved (Bou, 1974). A Bou-Rouch pump can be made for about $100 US, using materials available at any plumbing store. Buy a wellpoint, punch through the screening of all openings in the lowest 15 cm of the pipe, and using bathtub caulking, seal up the rest of the openings (i.e., those above 15 cm). A hand pump that pulls a large volume per stroke such as a hand-operated bilge pump from a boating/marine store or boating supplies from online sources are effective. These will need to be maintained, but these are durable, adaptable, and inexpensive. In the field, the pipe is driven into sediments with a 1 to 2-kg sledgehammer. Once at the desired depth, interstitial water containing the meiofauna is pumped into and through a sieve or sieve series (see also Chapter 25).

The method of Karaman-Chapuis is useful for sampling meiofauna in the water beneath gravel banks at the margins of rivers. It involves digging a hole into the gravel until the water level is reached, and then collecting the water as it seeps into the hole before pouring it through a sieve. A combination with Bou-Rouch sampling seems to work quite well. Initially, dig into the stream bank and sample using the Karaman-Chappuis pit, then hammer the Bou-Rouch pipe into the bottom of the pit and pump the pit dry.

Collecting meiofauna from mosses and other plant material can be performed using Baermann funnels (Fig. 14.4A), in which meiofauna migrate down the grid as the sample gets dryer under gentle heating. Organisms then sink into the funnel capped with a lower-end tap. A fraction of bottom water should be retrieved daily during one week and poured through a sieve to concentrate animals for live observation. Note that after each collection, filtered stream water should be gently added on top of the funnel using a wash bottle, until no air bubbles hamper the migration of animals through the grid.

A turkey baster or similar suction device is particularly useful for qualitatively sampling the upper layers of muds and silts that can be sucked up and placed directly into a bucket. Then, by bubbling air into the bucket with fresh fine sediment the meiofauna will have a tendency to float on the water surface. Animals can then be collected by skimming with a fine mesh net, or by using blotting paper with subsequent rinsing. These and additional techniques are discussed by Higgins and Thiel (1988).

14.2.2.2 Quantitative Collection of Samples

A vast array of approaches has been used to sample meiofauna from different habitats in a quantitative way. We will mention only a few of these approaches here. First, we will present some approaches to collect the meiofauna dwelling on epibenthic surfaces. For meiofauna dwelling in epilithic biofilms coating cobbles, the investigator should carefully slide a cobble into a plastic bag underwater. Then, flat surfaces can be scraped with a toothbrush and rinsed with a wash bottle over a sieve. The scraped area should be measured afterward by taking scaled photographs of cobbles (Fig. 14.4B). For sampling biofilm-dwelling meiofauna from localized areas or from unmovable hard substrates, use a brush-sampler by scraping a known area of biofilm underwater (Fig. 14.4C, full details in Peters et al., 2005). Note that “homemade” brush-samplers can be crafted by gluing a toothbrush head onto the piston crown of a 30-mL syringe.

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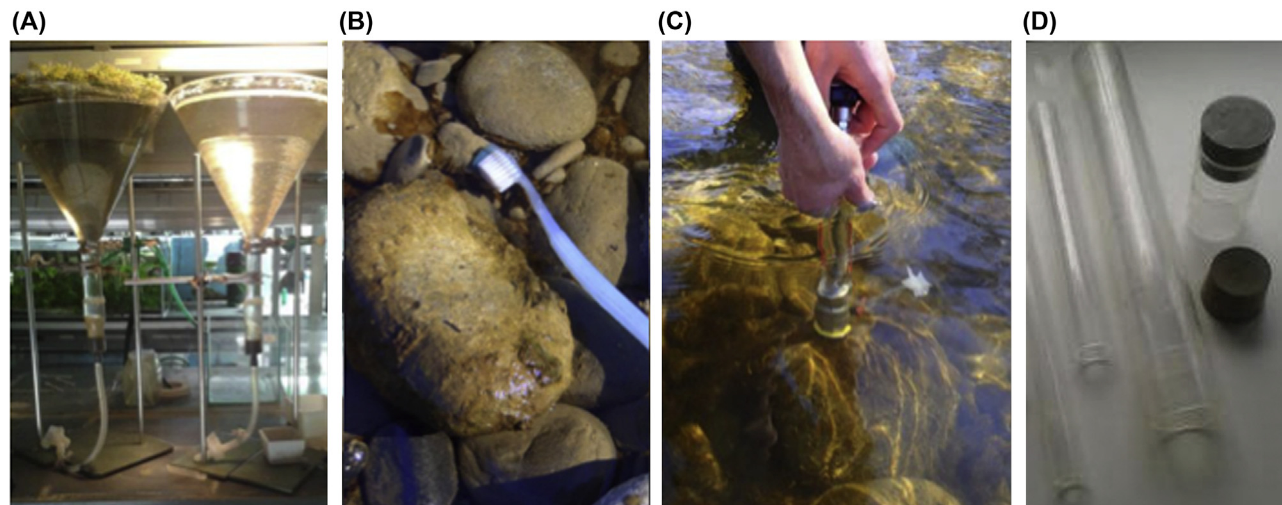

FIGURE 14.4 Examples of procedures to sample meiofauna. See in text for more details. (A) Baermann-funnel extraction. (B) Biofilm area scraped from a cobble using a toothbrush. (C) scraping and sucking biofilm underwater using a brush-sampler. (D) various corers with corks to sample sand and mud-dwelling fauna.

To collect meiofauna dwelling in complex/soft organic substrates (e.g., mosses, macrophytes, woody debris, leaf litter), the investigator should carefully slide samples underwater in a plastic bag and relax meiofauna with $MgCl_2$ (explained in greater detail below). After stirring, samples can be poured over a sieve, and all coarse material should be gently washed over the sieve with a water jet, and then put aside, dried, and weighed to express meiofaunal abundances per gram habitat dry weight.

To collect meiofauna dwelling on interstitial particles of the stream substratum, the best and simplest sampling device is a corer made of PVC or clear acrylic pipe (Fig. 14.4D). If sampling is to be relatively shallow, then a corer made out of a cut-off 30-mL syringe works quite well. The investigator should stand downstream of the sampling site to prevent disturbance of the area to be sampled. The corer should be inserted into the sediment in an area that has not been disturbed. If the sediment is fine (i.e., muds and silts), it is possible, after the corer has been inserted into the substratum, to place a cork in the top of the corer before withdrawing the corer from the streambed; this will prevent sample loss. In all other types of substratum, the investigator will need to push his/her hand down under the core bottom before pulling the corer out of the streambed. Samples collected in gravel or sand substrata should be acquired to a depth of at least 10 cm into the bed. In muds and silts, coring can be shallower (1−5 cm), because the depth of oxygen penetration is generally less than in sands and gravels. Particularly in silts and muds, cores can be sliced to investigate the vertical distribution of interstitial meiofauna.

For sampling deeper in sandy beds, a standpipe corer (Williams and Hynes, 1974) works well. This corer allows one to collect intact samples from discrete depths in the streambed. For streambeds that simply cannot be cored, a Bou-Rouch pump should be used, and has the advantage that the pipe component of the device can be left in place between sampling dates. The disadvantage of pump sampling is that (1) animals are often damaged, and (2) it is difficult to quantify samples based on differences in the resistance of animals to the pumping flow (Tanaka et al., 2014). In addition, one can report numbers of animals per volume of water pumped, but it is almost impossible to know from what area of the streambed this water originated, or how far out into the substratum the device is effectively sampling. Another alternative is to use in-situ freeze-coring devices. Bretschko (1990) used this technique quite effectively and minimized faunal avoidance of the sampler by electroshocking the area prior to sample collection, which stuns the animals so they do not migrate away from the freezing surface.

14.2.3 Sample Preservation

Once samples have been retrieved from the field, they should be transferred to a sample container holding several mL of 6% $MgCl_2$ anesthetic (73.2 g/L). The sample should be stirred and left to sit for c.5 min, after which it should be rinsed through a sieve. The contents of the sieve should then be rinsed back into the sample container using deionized water and a wash bottle. Several mL of 10% buffered Rose Bengal-formalin solution should then be added to the sample. Rose Bengal (1 g/L in 10% formalin) is a protein stain that greatly facilitates microscopic sorting. Some animals will stain in 15 min but

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many require 48 h for optimal staining. A final concentration of 4% buffered formalin in the sample is enough for long-term preservation of most hard-bodied (i.e., chitinous) meiofauna. It has to be noted, however, that formalin is highly carcinogenic, degrades most soft-bodied meiofauna (e.g., microturbellarians), and can hamper identification, since it causes some specimens to undergo body distortion. For appropriate counting and identification of soft-bodied meiofauna, it is recommended that samples should be kept fresh, and fauna processed alive. Formalin preservation is also discouraged for some analyses of meiofaunal diet based on gut pigment contents, stable isotopic signatures, or fatty acid composition of tissues; if possible, "live-processing" or preservation by deep freezing is preferable for all those dietary analyses.

Ethanol preservation is a good alternative to formalin for soft-bodied meiofauna. An initial killing solution of 40% ethanol should be added, followed by additions of solutions of graded ethanol concentrations during a period of a few hours, to reach a final ethanol concentration of 80%. The addition of a small amount of glycerol will prevent complete dehydration of the sample. For histological studies, specimens can be stained if placed for 24 h in Bouin's fixative prior to storage in ethanol, but researchers should note that this step is incompatible with fluorescence-based imagery and DNA analyses. If specimens are required for DNA analyses, they should be placed in absolute ETOH for long-term storage.

14.3 SPECIFIC METHODS

14.3.1 Basic Method 1: Observing the Living Meiofauna and Their Adaptations

It is useful for those new to the field of meiobenthology to familiarize themselves with the dominant members of the species assemblages and their morphologies, locomotion, modes of reproduction, and feeding. We recommend watching the documentary "Life Between Grains," which was produced in 2012 by the Marine Biology Center of the University of São Paulo and is available on YouTube. Although dealing with marine meiofauna, this documentary is easy on the eye and represents a handy introduction to the following practical exercises.

1. Use sand, mud, leaf, or algal material collected from the field and kept cool following the protocols described above. To observe living fauna in their natural habitat, suspend collected substratum material into water, and pour the water through a sieve. A following aliquot of this sample can be transferred to a Petri dish for direct observation using a stereomicroscope at a magnification between ×20 and ×50. As the sample begins to reach room temperature, the animals will become more active and more easily observable. "Cool," fiber-optic light is preferred, but "warm," transmitted or reflected light is acceptable.
2. Scan the sample and identify fauna to major taxonomic levels. If observation is made difficult by copious amounts of large particles, one should use a swirl-decant or baermann-funnel procedure (Fig. 14.4A) to better extract the living fauna. While identification of meiofauna to genera or species is no easy task, the investigator should be able to recognize the major groups using the stereomicroscope: rotifers, chironomid larvae, harpacticoid copepods (adults and nauplii), oligochaetes, and nematodes. Depending on the material, gastrotrichs, tardigrades, cladocerans, ostracods, microturbellarians, water mites, and various insect larvae should be found. A copy of Smith (2001), Thorp and Covich (2009), Merritt et al. (2008), or other freshwater invertebrate identification key will greatly facilitate this exercise.
3. Note the general differences in taxa abundance, body shapes, and body sizes among different types of habitats. An easy way to estimate body size is to stick millimetric paper beneath the Petri dish, and to note maximum body length for each individual at a glance or using microphotographs. Animals from sand will probably contain small, slender representatives of each taxonomic group and should have more diverse, but smaller, fauna than those in mud or litter samples.
4. Note the specific modes of meiofauna locomotion. Mites and tardigrades waddle along grains using their four pairs of legs, which are equipped with tiny claws. Microturbellarians and gastrotrichs glide onto hard surfaces. Nematodes wriggle and move between grains through alternate pushing and bending; some species are even able to "jump" by sudden relaxation after bending. Many species exhibit other adaptations for a benthic existence, including adhesive organs such as the "toes" of rotifers. These structures are usually found by mounting a specimen on a glass slide and observing it under higher magnification using a compound microscope (see Step 7 below). Adhesive organs are believed to reduce flow erosion from epibenthic surfaces and/or help displacements within the highly mobile sandy substratum.
5. Copepod movement is also very interesting to observe. Isolate copepods from different habitat types—for example, some from sand, some from mud, and some collected from litter samples. Note the difference in appendages and body width. The sand dwelling, interstitial copepods, especially the harpacticoids, should have smaller legs than the copepods from the other substrata. The legs of these interstitial animals can closely adhere to the narrow or bullet-shaped body. Compaction of the legs close to a fusiform body makes it easier for the animal to "glide" among the

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interstices of sand. The mud-dwelling copepods will generally be larger than the interstitial forms and will have more robust bodies and stout appendages that are used to help the animal push through mud while it burrows. The leaf- or algae-dwelling copepods will have a large, often somewhat flattened cephalothorax and appendages, especially the first leg, because they spend their lives clinging and swimming among structures.

6. Depending on the time of the year, individuals in various states of reproduction should be found. Copepods are often found *in copula* within samples. The male grasps the female's urosome with his first antennae and will eventually pass a sperm sac to the female. Eggs (usually 2−10) should be seen attached ventrally to the female's abdomen. Cladoceran eggs should be visible through the body wall. In nematodes, it is possible to distinguish males from females by the presence or absence, respectively, of copulatory spicules that are used in sperm transfer. Some nematode species are hermaphroditic and can lay or bear their self-fertilized eggs until they hatch. Sexual reproduction is rarely seen in rotifers; yet eggs should be seen developing through the body wall. Freshwater meiobenthic oligochaetes and turbellarians reproduce asexually or sexually, so individuals in various states of budding should be found in samples. The chironomids (informally known as nonbiting midges) are a quite diverse family of dipterans, and their larvae are present in freshwater habitats worldwide. Early instars of chironomids contribute significantly to the density and biomass of the meiofauna. The larvae of chironomids pass through numerous instars as they grow and mature, pupating and emerging at various times of the year. Adult chironomids are commonly observed in mating swarms near running-water ecosystems. The adults live a brief aerial existence before they mate and fly back to the stream to lay eggs. Note that for chironomids, identification to genus- or species-level is much easier when based on the morphology of pupae or adults. Floating pupae can be collected by skimming off the water surface with a fine sieve. Adults can be collected using a butterfly net or emergence traps (see Chapters 15 and 21).
7. If higher magnification is desired for further examination, specimens should be transferred to a drop of water on a glass slide, a small piece of hair or wax inserted as a spacer, and a cover slip placed on top. Anesthetics (e.g., $MgCl_2$, club soda, or sparkling water with a high CO_2 content) should be added to the Petri dish sample to slow animals. Anesthetization also facilitates the transfer of specimens to slides using meiofaunal-sized tools like "worm pickers", "mouth-pipettes" or Irwin loops. The latter are small, wire, inoculating loops used in bacteriological work. Inexpensive "mouth-pipettes" can be made by drawing out Pasteur pipettes (i.e., the pipette is heated and the tip lengthened to reduce the internal diameter of the tip to c.300 μm), before fixing a flexible tube (the drinking straw) to the tail of the pipette. Mouth-pipettes are ideal for handling cultures and for quickly sorting out and washing important numbers of individuals from a bulk sample. However, only use mouth-pipettes with nonpreserved samples, or after rinsing thoroughly any preservatives from the samples. "Worm pickers" are easily made by gluing an eyebrow hair on the tip of a Pasteur pipette. They are especially useful to transfer and arrange worm-shaped meiofauna on microscope slides. Once animals are on a slide, they can be slowed down by simply placing a tissue at the edge of the cover slip and drawing out just enough of the mounting water to squeeze them a bit.

14.3.2 Basic Method 2: Extraction, Enumeration, and Identification of Preserved Meiofauna

1. To facilitate quantification and microscopic identification, animals should be extracted from substrata using a variety of quantitative techniques. When the substratum is coarse (i.e., sand, gravel, cobbles), the easier technique is a simple swirl-decant procedure (see details above). However, if formalin was used as preservative, be sure to rinse the samples well under a fume hood before any handling, and note that if the swirl-decant technique is being used for a quantitative study, the technique must be "calibrated" for each sediment type. For instance, using a dissecting microscope, the sorter should examine the sediment remaining in the bucket after six decantations to be certain most of the animals were removed.
2. For sand and fine sediments, either the entire sample must be microscopically examined or a more complicated extraction procedure used. Many meiobenthologists employ a density gradient technique for quantitative extractions. Here, the preserved sample is rinsed into a centrifuge flask or tube, depending on the amount of substrate to be extracted. A rule of thumb is that the amount of sample to be extracted should not exceed a fifth of the container's volume. Then, the tube is filled with water and the sample centrifuged at high speed (c.1500 g) for 5 min to remove preservatives and excess water. The supernatant is poured through a sieve, with the sample "pellet" left in the tube. Occasionally, a few animals will be retained on the sieve, so the contents of the sieve should be rinsed into a jar to be merged with any further extracted fauna. The centrifuge tube containing the pellet should then be filled 80% with a colloidal silica solution (Ludox-TM distributed by Sigma−Aldrich at c.$40 US for 1 L). If necessary, the specific gravity of the solution should be set at 1.14 g/cm^3 by adding distilled water. After capping the tube, the sample

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should be stirred thoroughly for 3 min to be certain that the entire pellet and associated animals are well suspended in the solution. The sample should then be centrifuged at a lower speed (800 g) for 5 min. Most of the animals will be floating in the silica supernatant, whereas inorganic particles—more dense than the silica solution—will sink. Pour the supernatant through the sieve and, using a wash bottle, carefully rinse the sides of the tube and the cap, but not the sediment pellet, onto the sieve. To reduce costs substantially, the Ludox solution passing through the sieve can be collected and recycled to a specific gravity of 1.14 g/cm^3 by gently boiling excess water in the solution. This density gradient technique is compared to other extraction procedures in Pfannkuche and Thiel (1988). Note that Ludox extraction is very effective and reliable and that only one round of centrifugation generally extracts more than 90% of the fauna from the sediment. After extraction, the remaining sediment pellet should be rinsed over stacked sieves, and size fractions should be dried and weighted to obtain the sediment grain-size distribution of the sample from which meiofauna have been extracted.

3. Once meiofauna have been extracted from the sediment, a subsample (see Fleeger et al., 1988 for a discussion of subsampling) should be placed in a Petri dish. The bottom of the dish can be "gridded" by simply sticking a patch of millimetric paper beneath the dish. This will enable the user to make body-size measurements for each specimen while counting (see Table 14.1). Meiofauna should be counted from the whole dish under a stereomicroscope at c.20× magnification. Note that higher magnification is desirable, since many of the rotifers, nematodes, gastrotrichs, and tardigrades are very small and could require zooming for identification. Relevant estimation of densities can be assumed after >200 individuals have been counted from subsample(s). Use Table 14.1, as a template for quantitative assessment of meiofauna dwelling in a given habitat. To help derive taxa biomass from body-length measures, use the online Worksheet.
4. For accurate identification, the animals should be transferred to a drop of water on a glass slide and viewed with a compound—or preferably, a phase contrast—microscope. Hulings and Gray (1971) provide a more lengthy discussion of mounting techniques, but note that semipermanent slides can easily be made by mounting specimens directly from

TABLE 14.1 Sample data sheet for identification, measurement, and enumeration of meiofauna.

Sample Site:			Date:			Sorter:		
Total Sample Volume or Weight:						**Subsample Volume or Taxon:**		
Taxa	**Number of Indiv. in Body-Length (mm) Class**					**Total Number in Subsample**	**Total Number in Habitat (e.g., Indiv./g or cm^2)**	**Contribution to Meiofaunal Assemblage (%)**
	<0.5	**0.5–1**	**1–1.5**	**1.5–2**	**>2**			
Nematodes								
Rotifers								
Harpacticoid copepods								
Chironomids								
Ostracods								
Cladocerans								
Water mites								
Gastrotrichs								
Tardigrades								
Microturbellarians								
Oligochaetes								
Other:								
Total meiofauna								

Indiv., Individuals.

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water, alcohol, or formalin into the CMC series of mounting media (available from the Masters Chemical Co., Elk Grove, IL), which also clears the specimens nicely. Ring the cover slip with clear nail polish after a day or two, and the slide can last for years. Preparing permanent slides involves a transfer of specimens in glycerol. However, an abrupt transfer from aqueous media to glycerol can cause some specimens to shrink. To avoid this, Seinhorst (1959) proposed a gradual transfer in glycerol through successive evaporation of two water/ethanol/glycerol solutions in an open concave watch glass (solution A 79/20/1; solution B 0/95/5). After solution A has evaporated for a few days at room temperature, and taking care that specimens stay in the bottom of the watch glass, add solution B, and the specimen will end up in a drop of pure glycerol after about a week.

5. Identification to major taxonomic groups is not difficult (e.g., Fig. 14.1). Most meiofauna can even be readily identified to the level of family using common freshwater invertebrate keys (see Smith, 2001). Identification to genus or species can be difficult, and often requires special techniques, strong experience, and an updated knowledge of the primary taxonomic literature. Note that the Handbook of Zoology database is available online and provides an updated in-depth taxonomic overview of most animal taxa, as well as useful references for investigators interested in the species identification of meiofauna. Investigators may also find support by contacting specialized taxonomists and through attending workshops and meetings of the International Association of Meiobenthologists (www.meiofauna.org).

14.3.3 Advanced Method 1: Molecular Approach to Meiofauna Taxonomy and Species Distribution

1. Meiofauna are ideal animals for studying recolonization processes in streams because they are everywhere, easy to enumerate, and recover rapidly following disturbances. However, identification to the species level can be a long and arduous task for some taxa. Further, there is cryptic diversity (i.e., two or more different species sharing a similar morphology). Cryptic diversity seems extensive and is likely underestimated in meiofauna (Tang et al., 2012; Derycke et al., 2013; Ristau et al., 2013). More than a decade ago, DNA barcoding was proposed as a promising approach to estimate species' diversity and help study species' dispersal, phylogeography, and resilience. Now, molecular analyses are increasingly accessible, providing a high throughput of sequences to which new DNA barcodes can be compared. Nevertheless, questions still remain about criteria for species delimitation, and multiple genetic markers should be combined with morphology and species-distribution modeling to strengthen the conclusions of DNA surveys (Carew and Hoffmann, 2015).
2. Relatively cheap high-throughput molecular techniques are now widespread in ecological and evolutionary research for the scoring of Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphisms (SNPs), and microsatellites from environmental samples. A critical step, however, remains the extraction of DNA from bulk samples. For extracting meiofaunal DNA from sediment samples, a simple and cheap method is the hot sodium hydroxide and Tris (HotSHOT) extraction initially developed by Montero-Pau et al. (2008) for barcoding eggs of crustaceans and rotifers diapausing in lake sediment. This method yields good extraction results from fresh and ethanol-preserved sediment material. After extracting meiofauna from the sediment using Ludox flotation (see Basic Method 2, above), specimens are sorted, washed, and transferred into individual microtubes containing 50 μL of an alkaline lysis buffer (disodium EDTA 0.2 mM, NaOH 25 mM, pH 8). Crush armored individuals against the sides of the tube to improve extraction efficiency. After 30 min of incubation at 95°C, cool the tubes on ice before adding 50 μL of the neutralizing solution (Tris−HCl 40 mM, pH 5). Stir well using a vortex mixer. A few μL of the solution containing the genomic DNA of a single meiofaunal specimen can be used for PCR-amplification.
3. Compared to marine habitats, only a few molecular surveys of meiofaunal populations are available from freshwater habitats. However, specific methods and/or integrated approaches developed primarily for marine systems are applicable to freshwater meiofauna as well (e.g., De Ley et al., 2005; Bhadury et al., 2006; Derycke et al., 2010). Recently, Ristau et al. (2013) studied nine postglacial European lakes in the first extensive molecular survey, exploring cryptic species diversity and genetic population structure in a widespread freshwater nematode morphospecies: *Tobrilus gracilis* (Bastian, 1865). After specimen identification to species under the microscope, individual DNA was extracted, amplified, and analyzed for gene fragments of cytochrome *c* oxidase subunit I (COI), large ribosomal subunits (LSU, or 28S), and small ribosomal subunits (SSU, or 18S) (see details in Ristau et al., 2013 for primers and PCR conditions). After analyzing the sequences, a remarkable genetic differentiation of the population emerged, even at a small geographical scale, supporting the existence of three genetic lineages within *T. gracilis* (Tg I−III, see Fig. 14.5). At a larger geographical scale, the three cryptic lineages exhibited notable patterns of sympatry, suggesting ecological specialization and/or recent recolonization events. In river catchments, we know virtually nothing about meiofauna genetic differentiation and gene-flow patterns.

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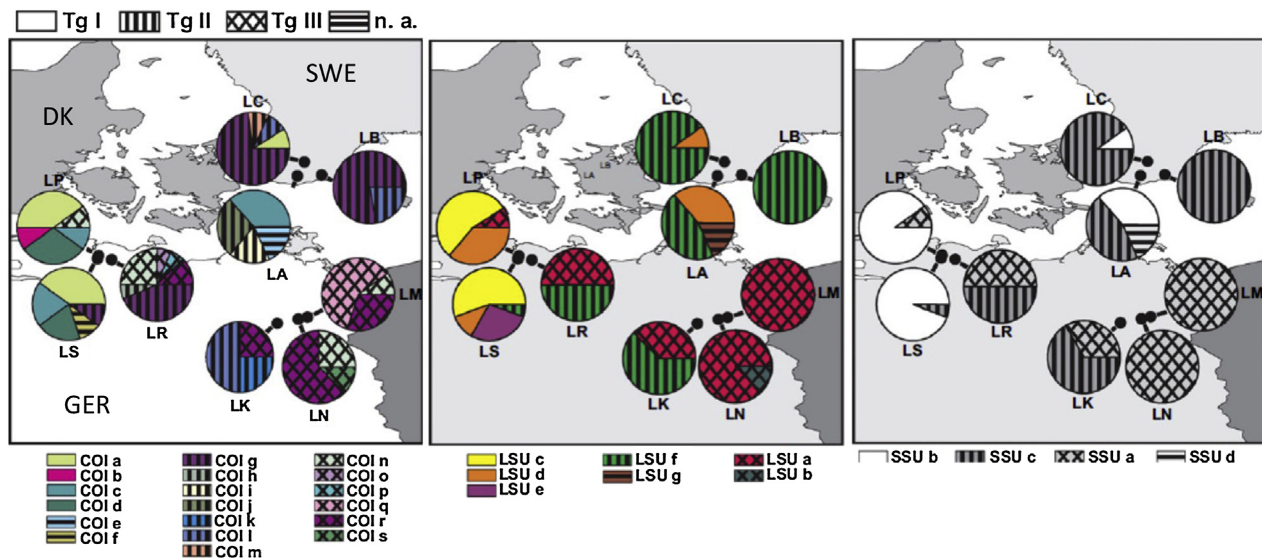

FIGURE 14.5 Spatial Distribution of the nematode *Tobrilus gracilis* haplotypes, based on, from left to right: cytochrome *c* oxidase subunit I (COI), large ribosomal subunits (LSUs) and small ribosomal subunits (SSUs) gene fragments. *Pie charts* represent the contribution of haplotypes found in a given lake. Haplotypes are grouped according to cryptic lineages Tg I–III. *From* Ristau et al. (2013).

14.3.4 Advanced Method 2: Establishing Laboratory Cultures and Determining Effects of Toxicants on Development and Reproduction

Many meiofaunal taxa are highly amenable to laboratory culture and experimentation. Their small size and relatively short generation-times mean that they can be maintained in high numbers on a small scale. Also, measures that relate to fitness can be obtained rapidly. Here, we describe a laboratory protocol for culturing stream harpacticoid copepods, and we describe how to use development times and reproductive success as surrogate measures of the influence of a toxic stress. Cultures should be established a few months before experimental trials, and researchers are highly encouraged during this establishment to practice handling, observing, and identifying the different life stages. For other meiofaunal taxa, many specific cultivation techniques can be found in the literature: for nematodes, Moens and Vincx (1998), Muschiol and Traunspurger (2007), and Muschiol et al. (2009); for gastrotrichs, Bennett (1979); for bdelloid rotifers, Ricci (1984); and for tardigrades, Altiero and Rebecchi (2001). The aim of this section is not to present exhaustive cultivation techniques, but rather to give basic steps and a simple framework for using meiofauna from cultures in standard toxicity assessments.

14.3.4.1 Establishing Laboratory Cultures and Measuring Development and Reproduction

1. A sample of meiofauna should be collected as outlined above, observed live in the laboratory (Basic Method 1), and harpacticoid copepods (females with eggs or mating pairs) extracted using a mouth pipette. Individuals (or pairs) should be placed into separate small containers (e.g., in 12-well plates). Although chances are high that there will be several species at the site sampled, sorting animals in this way should increase the likelihood that only one species is cultured.
2. Copepod cultures can be initiated by placing individual females or mating pairs in large Petri dishes containing growth medium (e.g., ASTM, 1980) and leaf fragments that have been preconditioned in stream water for two weeks. Ideally, cultures and trials should be maintained at a constant temperature with a day-to-night light regime and the water exchanged every two to three days with filtered stream water. Cultures will need to be split if densities get too high.
3. At 20°C, development times for many harpacticoids are 15–20 days. Expect longer development for trials carried out at lower temperatures. Developmental trials should be started using nauplii obtained from ovigerous copepod females isolated from cultures. Nauplii (N) should be maintained individually in 12-well plates containing 2 mL of medium and a 4 mm-diameter conditioned leaf disc.
4. Copepod survival and the presence of molted exuviae in wells should be recorded daily (Table 14.2) and used to calculate the duration (1) of the combined naupliar stages (Dn), (2) of the individual (C1, C2, C3, C4, C5) and combined

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TABLE 14.2 Measuring the development and reproduction for meiobenthic copepods. A mark should be placed in the appropriate column of the table if that stage is present. Each well starts with one copepod nauplius (N). Transition to first copepodite (C1) stage is dramatic and obvious in copepods, following stages can be inferred through the presence of empty exuviae in the wells. Each time an exuviae is found, it should be removed from the well. Dn = $\sum$ of hours in C1 (e.g., Date 5 − Date 3 in the example below); Cn = $\sum$ of hours in C1−C5 (see text).

Well #:	Species:		Treatment:		Temperature:	
Copepod Life-Stage						
Date	N	C1	C2	C3	C4	C5
Date 1	×					
Date 2	×					
Date 3		×				
Date 4		×				
Date 5			×			
Date i						

copepodid stages (Dc), and (3) of the total development time (i.e., from the first naupliar stage to the adult stage) (Brown et al., 2003).

5. The Dc/Dn ratio can be used to investigate a standard development index for the species; the duration of larval stages allows one to test for equiproportional development (ED) (see following). Reproduction trials should be conducted using newly mated pairs from cultures—that is, mating pairs where the female has no egg sac. Pairs should be placed in 12-well plates and be maintained as for nauplii, but with a larger (10 mm-diam.) leaf disc. Daily observations of the presence of egg sacs and nauplii should be made to calculate the embryonic development time (i.e., the time from egg sac release to hatching) and the number of broods per female.
6. Transfer pairs to a new 12-well plate (with new media and leaf disc) every 7 days, and count remaining nauplii (those from separate broods can be distinguished by size) and unhatched eggs. This allows the investigator to calculate embryonic development times, numbers of broods per female, hatching success, and total offspring production.

14.3.4.2 Performing Single-Species Toxicity Tests and Monitoring Effects at the Community Level

Alterations of the development and reproduction of meiofauna are increasingly used as proxies for the effects heavy metals, pesticides, and antibiotics can have on resident benthic communities (Chandler and Scott, 1991; Chandler and Green, 1996; Brinke et al., 2010; Faupel and Traunspurger, 2012; Monteiro et al., 2014; Hägerbäumer et al., 2015). Here, we will briefly describe how to perform toxicity tests on a single cultured species (see above), and then present some potential micro- and mesocosm devices to study long-term effects of toxicants in complex benthic communities.

1. The sublethal effects of toxicants can be investigated by comparing development and reproduction in treatments containing varying levels of toxicants released by human activities that are likely to affect stream ecosystems (e.g., trace metals, pesticides, antibiotics, salts from road deicing, etc.). As a practical example, one can use the deviation from ED as a proxy for toxic effect in copepods. Without toxic stress, the proportion of total development time spent in each molt stage is constant. Disrupted ED is predicted when toxicants affect developmental processes such as molting (Brown et al., 2003).
2. Aquatic sediment microcosms (c.0.5-L sediment) are useful to assess the effects of toxicants on complex meiofaunal communities, as they are small enough to provide relevant replication at a convenient scale, but still large enough to

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house realistic meiofaunal species assemblages. Container designs are various: experimental streams, aquaria, sediment cylinders, or boxes, all provisioned (or not) with a single or cocktail of toxicants (see review of Hägerbäumer et al., 2015 and references therein). With meiofauna, experiments can run several years, but can also be monitored over a few months, which is enough to observe significant effects over several generation-times. Changes in species richness, species evenness, functional diversity, and secondary production between controls and contaminated treatments indicate effects of toxicants at the community level. Microcosms are also useful when one wants to track the fate of chemicals along food chains, or when one wants to discriminate tolerant versus sensitive species in order to develop indicators for biomonitoring.

14.3.5 Advanced Method 3: Determining Trophic Relationships

The meiofauna are enormously diversified in their feeding habits and include suspension feeders, bulk deposit feeders, biofilm scrapers, selective grazers of benthic algae, grazers of vascular plants, highly specialized predators, generalist predators, and parasites. As small organisms, meiofauna also are prey for many macroinvertebrates and juveniles of benthivorous fishes (see e.g., Rundle et al., 2002; Majdi and Traunspurger, 2015 and references therein). There are several simple or more sophisticated ways to learn about the trophic relationships of meiofauna. The simplest way is direct observation of size, morphology, feeding behavior, and gut contents to evaluate the nature of the trophic interactions that a meiofaunal community develops with other stream biota. Laboratory and in-situ enclosures, or "cafeteria" experiments can be used to investigate consumptive and nonconsumptive interactions between predators and their prey. Finally, other more sophisticated approaches are based on the fate of trophic tracers to measure the ingestion and the assimilation of food. Recent methodological advancements are presented briefly at the end of this section.

14.3.5.1 Observation of Morphology, Movements, Feeding Behavior, and Gut Contents

1. It is possible to estimate the diet of an animal by observing the size and morphology of its mouthparts. For example, nematode species from the genus *Prionchulus* show large armed buccal cavities allowing them to prey on smaller nematodes (Fig. 14.6). In contrast, nematode species from the genus *Plectus* show a minute and unarmed buccal cavity allowing them to prey only on microscopic prey like bacteria (Fig. 14.6). Dorylaimid nematodes show hollow spears and muscular pharynxes for piercing their prey and pumping out their cellular contents. This enables these "suction feeders" to prey on plant material or animals much larger than themselves (e.g., fungal hyphae, plant roots, macroinvertebrates). Formal schemes have been developed for relating the structure of mouth to the diet of meiofaunal organisms, such as in nematodes (e.g., Yeates et al., 1993; Moens et al., 2006; Traunspurger, 1997, 2009, 2014) (see Fig. 14.6). However, feeding opportunism seems widespread in meiofauna, and caution must be taken when inferring diet from morphological data only.

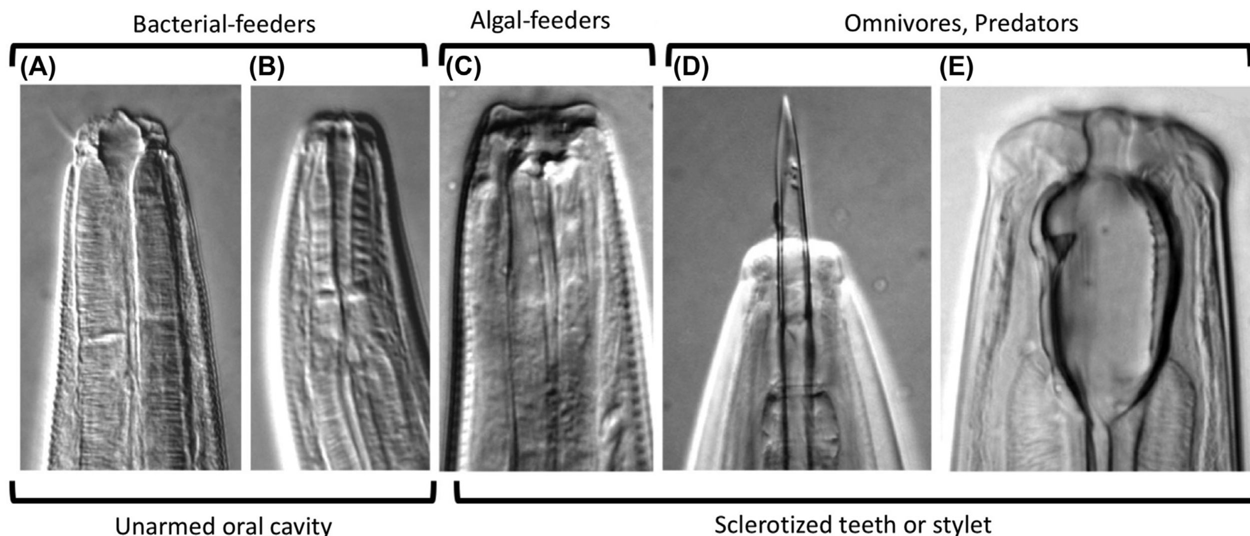

FIGURE 14.6 Illustration of freshwater nematode-feeding types classification proposed by Traunspurger (1997). (A) and (B) Deposit-feeders with minute unarmed mouth only enabling engulfment of prey in the bacterial size range [(A) *Daptonema*, (B) *Plectus*]; (C) Epistrate-feeders with small teeth enabling piercing/cracking of diatom frustules (*Punctodora*); (D) Suction feeders with sharp retractable stylet enabling feeding on inner contents of a variety of prey (*Dorylaimus*); and (E) Chewers with large mouth armed with jagged teeth enabling engulfment of diatoms, protozoans, and other meiofauna (*Prionchulus*).

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2. Prepare a thin layer of Agar in 9 cm-diameter Petri dishes (c.15 mL of a 0.8% Agar solution per dish, autoclaved and poured while still hot). Wait for the Agar to cool, then simply inoculate with 15 mL of stream water and spread about 1 g of a meiofaunal subsample in the plate (see Basic Method 1 above), then seal the dish with parafilm. The investigator can repeat inoculation in many dishes using meiofaunal samples from different habitats. After a few days at ambient temperature, a diverse fauna and flora (i.e, diatoms, protozoans, rotifers, nematodes, copepods) will emerge and colonize the plates. Some species can thrive there for months. These "spot plates" are a first step to observe the feeding behavior of meiofauna (e.g., Moens and Vincx, 1997). Under the steromicroscope, try to identify the different feeding strategies (filter feeders, predators, diatom "crackers", generalist engulfers, etc.). The investigator should follow a single specimen for a while, and keep record (with camera or a voice recorder for instance) of frequencies of encounters with prey, successful attack rates, size of the prey in relation to the size of the predator, potential escaping behavior of the prey, etc.
3. Most meiofauna are so transparent that ingested items can be observed directly in their digestive system. Observations are possible either on living or preserved individuals mounted on microscope slides. If living specimens are to be observed, the investigator will likely have to slow them down by squeezing them under a cover slip or anesthetizing them with $MgCl_2$ or CO_2. Preserved specimens should be cleared in carboxymethyl cellulose (CMC-mounting media), and then observed under a compound microscope. Search for ingested detritic particles, diatoms' frustules, rotifers' loricas and jaws (mastax), crustaceans' carapaces and appendages, oligochaetes' setae, insects' head capsules, and all other recognizable hard structures that should persist in predators' guts. It should be helpful to examine a sample of sediment and other animals from the same site to aid in identifying the bits and pieces of food found in guts. Note that the gut contents are not a quantitative reflection of diet, because some food items are highly digestible and should disappear long before recalcitrant food items. Moreover, much material in the gut, especially detritus, should be unidentifiable. For instance, nematodes are very quickly digested after ingestion by chironomids (Ptatscheck et al., 2015), whereas frustules last longer, which distorts quantitative estimation of chironomids' diet in this case. Nevertheless, careful and extensive observations of gut contents have been successfully used to build detailed networks of meiofaunal trophic interactions (Schmid-Araya et al., 2002a).
4. Altogether, observational data should help the investigator assign each species a coarse diet (e.g., algivore, bacterivore, detritivore, omnivore, predator), and could even provide a more detailed list of prey, which should be used to construct a network of the trophic interactions (i.e., a food web) occurring in the stream ecosystem studied. FoodWeb 3D or Network 3D software can be used to map food web structure and to measure some food web metrics (www.foodwebs.org; Yoon et al., 2004; Williams, 2010).

14.3.5.2 Cafeteria Experiments

1. At the meiofaunal spatial scale, resource distribution can be very patchy and ephemeral. Species must quickly reach the best patches to forage before resources are gone. "Cafeteria" designs (or more formally: food-choice experiments) are well suited to investigating the attraction of meiofauna to specific types of food patches, which can suggest potential feeding preferences (Höckelmann et al., 2004; Weber and Traunspurger, 2013). This rather simple approach requires first cultivating or retrieving different food sources from the field (e.g., leaf disks, diatoms, *E. coli*).
2. Next, prepare large Petri dishes (15-cm diam.) filled on a flat and level surface with 40-mL autoclaved 1.5% Agar prepared with filtered stream water. Following a template placed beneath the dish, use a sterilized 10-mm cork borer to remove one central and eight equidistant peripheral plugs of Agar. Place test resources in four alternate peripheral holes. Alternate spaces are controls and should be kept empty.
3. Inoculate the central hole with one or several species of nematodes and copepods from your own stock cultures (see Advanced Method 2 and next experiment), or inoculate it with about 1 mL of a natural meiofaunal assemblage collected from a stream biofilm or litter habitat (see Basic Method 1, above). Gently add a thin layer of filtered stream water (c.20 mL). Incubate all replicate cafeteria plates at ambient stream or culture temperature and under a homogeneous light regime (or without light). Take care not to disperse the organisms while moving the plates.
4. Plates can be harvested after a few days. Use a 15-mm cork borer to remove a slightly larger area than the original patches. Store the plugs in a buffered 4% Rose Bengal-formalin solution. Identify and count the organisms that were foraging on each patch, including controls, and compare treatments to test for any significant attraction toward a particular resource.

14.3.5.3 Predator-Prey Functional Responses

1. Predation experiments on meiofauna can be performed by using various stream predators and prey. Hereafter, we present a simple protocol to test predation rates as a function of prey density to get insight into predator-prey functional responses. Here, we considered bacterial-feeding nematodes as prey, because they are often numerically dominant in meiofaunal communities and they are relatively easy to maintain in laboratory cultures.

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2. First, the investigator should prepare stock cultures of nematodes. The easiest way is to place a few grams of a natural sample (e.g., sediment, litter, moss) around a central *Escherichia coli* lawn of a slightly moistened, standard Nematode Growth Medium (NGM) culture dish. After a few minutes to hours at most, fast-moving bacterial-feeding nematodes are attracted by the *E. coli* lawn and will swim out of the sample. Migrating gravid individuals might be picked out gently using a worm picker and transferred individually to new culture dishes. After sealing the dishes with parafilm, they should be placed in the dark for a week at a constant temperature (e.g., 20°C) and checked regularly for the presence of juveniles, and eventually of other adults that could be used to identify the species currently in culture. Note that if needed, a lawn of frozen *E. coli* can be added to cultures to boost population growth. Stiernagle (2006) and Barrière and Félix (2014) provide more details on procedures for the culture and maintenance of *Caenorhabditis elegans*, which applies well (or may be slightly modified) in maintaining many species of freshwater bacterial-feeding nematodes in laboratory cultures. Population size will define the frequency at which cultures must be renewed (typically a few weeks to several months). For this, the easiest way is to cut out a 1 × 1-cm chunk of agar containing nematodes from the old culture and transfer it onto a new NGM dish. To reduce contamination of cultures, it is preferable to perform most maintenance under aseptic conditions (e.g., under a laminar flow hood). Algae-feeding and predatory nematodes are generally more difficult to keep in cultures, and therefore need specific culture settings (Moens and Vincx, 1998).
3. In order to obtain nematodes to be used as prey, rinse stock cultures with M9 medium over stacked 35- and 5-µm meshes to retain adults and large juveniles, respectively. The fraction passing through 5-µm meshes should contain mostly early nematode instars, which may be used as a smaller prey category (Ptatscheck et al., 2015).
4. Then, scoop some stream sediment or sample litter and stony habitats to find predators (see macrofauna sampling methods in Chapter 15). Tricladid flatworms, chloroperlid stoneflies, tanypodid chironomids, and other predatory dipter larvae, and even predatory nematodes and copepods, should be ideal predators for this experiment. Acclimatize the predators to laboratory conditions for a few days. Then starve the predators in filtered stream water for a couple of days before experiments.
5. Use 9-cm Petri dishes filled with 20 mL of filtered stream water as arenas (or smaller arenas for meiofaunal-sized predators). Arenas can be supplemented with a layer of autoclaved fine or coarse sediment to test the effect of habitat complexity on predator-prey interaction. Prepare multiple dishes, which will house a density gradient of nematode individuals retrieved from stock cultures. Use a mouth pipette to transfer—for example—25, 50, 75, 100, 200, and 400 adult nematodes into single arenas and replicate the entire gradient four to six times. After a few hours, check that the prey is evenly distributed in the dishes, then introduce single starved predators into each arena.
6. Some species of flatworms, copepods, tardigrades, and chironomids can feed voraciously on nematodes (Beier et al., 2004; Muschiol et al., 2008; Hohberg and Traunspurger, 2009; Ptatscheck et al., 2015), processing up to several nematode individuals per minute, so the investigator should adapt the timing of the experiment (c.2−3 h) to reflect this potentially high consumption rate. Stop the experiment by removing the predators and heating the plates for 10 min at 80°C. Add a few drops of Rose Bengal and directly count the remaining number of prey under a stereomicroscope.
7. Plot the number of prey offered initially versus the number of prey remaining in arenas, and fit a curve. Usually a Holling's Type II functional response curve emerges, in which the number of prey consumed increased with the density of prey offered, up to a plateau constrained by the prey-handling time. In other words, at very high prey densities, predators will spend almost all their time handling prey and very little time searching. It is interesting to investigate whether refuges provided by habitat complexity can affect prey searching time or handling time, and hence can modify the impact of predators on prey populations.

14.3.5.4 Field Enclosures

1. Predation on a complex meiofaunal community should better be examined in situ, using cages to include/exclude a given stream predator and investigate how their presence affects prey (and nonprey) populations. Indeed, the size of the cage and meshing must be selected to adequately confine the chosen predator. For instance, to confine macrofaunal-sized predators with litter-dwelling meiofauna, one can use fine mesh 10 × 10-cm bags containing leaf litter (Majdi et al., 2014). To confine/exclude carp juveniles used as predators, Weber and Traunspurger (2015) used much larger 70 × 70 × 100-cm cages made of 1-cm mesh wire attached to a metal frame and anchored in the sediment at a depth of 20 cm. They regularly sampled the meiofaunal community in and out of the cages during 4 months, and found evidence that carp juveniles exerted strong predation pressure on populations of sediment meiofauna.
2. Meiofauna move into the water and even disperse through the sediments in response to foraging predators and predatory cues (Shofner, 1999; Silver et al., 2002). Experimental work to examine escaping behavior has involved complex

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field experiments in which defaunated sediment was buried in areas surrounding predator enclosures. The meiofauna then moved into that defaunated sediment from within the cage sediment (see Palmer and Strayer, 1996 for basic methods). The defaunated sediment was later sampled to enumerate the meiofauna that have colonized it (Shofner, 1999). Laboratory experiments have also been performed in which meiofauna movements at the sediment−water interface and up into the water column have been quantified and shown to be significantly influenced by the presence of predatory cues. For example, fish produce chemical cues that meiofauna are able to perceive (Silver et al., 2002). An emerging technique, X-ray tomography, can reveal the movements of interstitial fauna within a sediment column. This technique is promising for investigating the movements of nematodes in response to various stimuli (Tom Moens, pers. com.), which may be, for example, the addition of predatory cues.

14.3.5.5 Use of Trophic Tracers and New Techniques in Trophic Ecology

1. Ecologists commonly use biochemical and isotopic tracers to follow trophic relationships in food webs (see Chapter 23). Typical fractionation of stable isotopes during the assimilation process already informs on the trophic position of organisms and the main resource they use to derive their biomass *in natura.* In addition, the proportion of a stable isotope can be artificially enriched in a particular resource to facilitate the measurement of its assimilation by consumers (e.g., Majdi et al., 2012b; Estifanos et al., 2013). However, stable isotopic studies rarely include meiofaunal organisms because of technical difficulties in analyzing isotopic ratios from small samples. Typically, the minimal N and C content required for detection in mass spectrometry is >20 μg per sample, which means cleaning and encapsulating hundreds of nematodes, gastrotrichs, copepods, or rotifers in small tin cups to measure any reliable isotopic ratio. This task is easier with larger mites, cladocerans, or chironomids, but still needs dozens of individuals and relatively intensive efforts to sort and clean them out of bulk samples. Indeed, such laborious sorting almost precludes species-level analyses. Nevertheless, mass spectrometry can be modified to allow the detection of isotopic ratios from smaller samples (Carman and Fry, 2002). A promising approach for measuring isotopic ratios in meiofauna is nanoscale secondary ion mass spectrometry (NanoSIMS). Although still rather expensive, NanoSIMS can reveal the spatial distribution of C, N, and S isotopic ratios at microscopic scales: in the tissues of nematodes for example (Tom Moens, pers. com.). NanoSIMS not only provides isotopic data at the level of single worms, but should also help researchers better map the mechanisms of isotope fractionation during food assimilation processes.
2. Specific biomarker pigments contained in green algae, diatoms, or cyanobacteria (see Chapter 12) can be tracked in the body of meiofauna through high-performance liquid chromatography (HPLC) and/or confocal laser scanning microscopy (CLSM) (Majdi et al., 2012c; Mialet et al., 2013; Occhipinti and Maffei, 2013; Kazemi-Dinan et al., 2014). If the sample is frozen using liquid nitrogen in the field, both techniques can provide measures of ingestion at a given moment so that diel patterns or selectivity in feeding can be investigated (see Fig. 14.7 for an overview of the procedure). Analyses of pigment composition can also reveal meiofauna-algae symbioses, and the use/conversion of ingested algal photoprotective pigments to reduce oxidative stress (Caramujo et al., 2012; Mialet et al., 2013).
3. DNA of ingested prey can persist in guts and even in fecal pellets, and thus can be amplified by simple PCR, using specific primers, to reveal the diet of meiofaunal organisms (methods described in Vestheim et al., 2005; Motwani and Gorokhova, 2013; Maghsoud et al., 2014). Interestingly, this approach can also reveal the consumption of meiofauna by much larger organisms (e.g., Read et al., 2006), which is still difficult to infer from traditional gut content observations due to the disappearance of most meiofaunal tissues during digestion. The recent development of next generation-sequencing metagenomics also allows investigating the microbial diet and gut microbiomes of meiofaunal organisms at an unprecedented resolution scale (Derycke et al., 2016). The most challenging issue is to control for the generation of chimeric reads in sequencing data (for more details, see Nichols, 2015). Nevertheless, emerging metagenomics approaches hold great promise for unraveling interspecific feeding selectivity and individual niche specialization in meiofaunal organisms (Derycke et al., 2016).

14.4 QUESTIONS

1. How might patterns in the species composition, abundance, and spatial distribution of meiofauna differ in fast-flowing cobble streams versus low-gradient, sandy streams with large amounts of woody debris? Consider not only differences in the taxa that might be expected in each stream but also in their dispersal abilities and feeding habits.
2. Given their vast differences in modes of locomotion and reproduction, you might expect meiofauna to differ greatly in their dispersal abilities. Which taxonomic group(s) would you expect to have the greatest dispersal potential? The lowest potential? Why?

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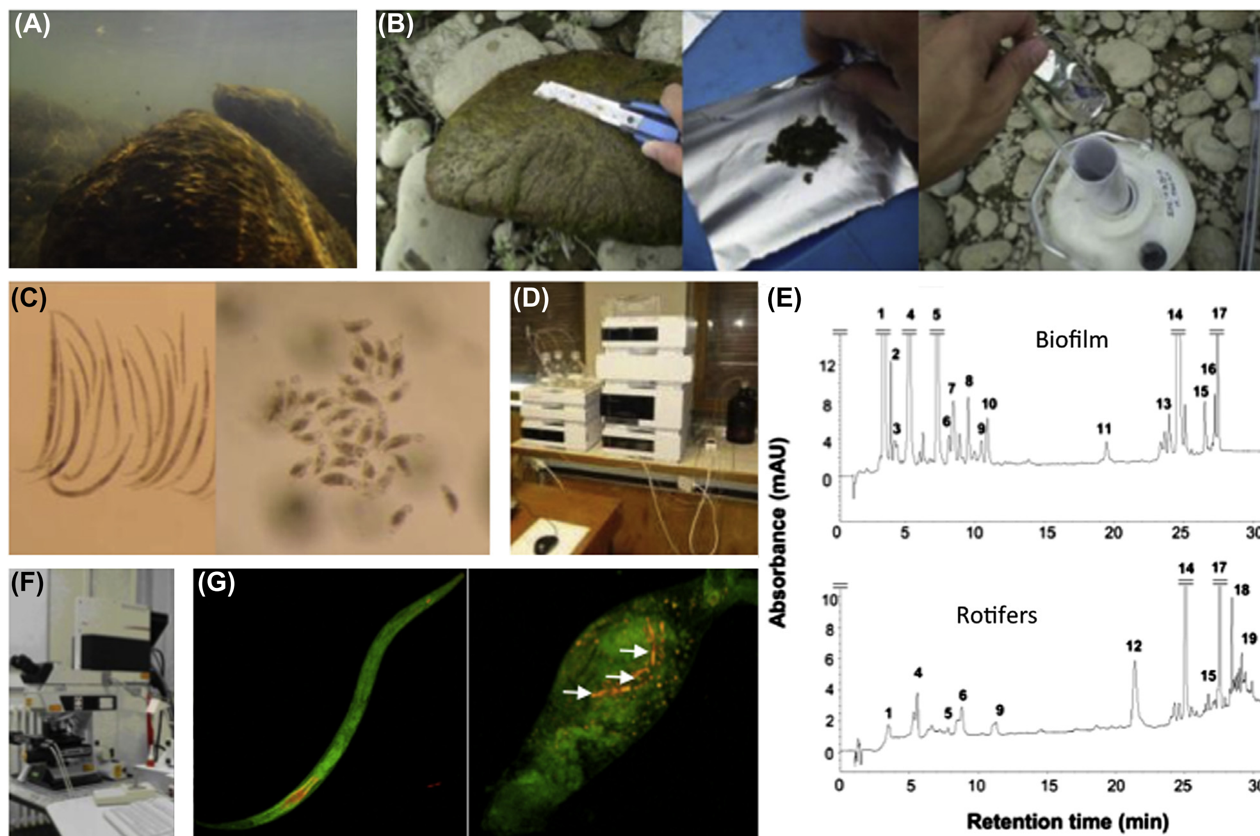

FIGURE 14.7 Illustration of procedures for gut pigment analyses of biofilm-dwelling nematodes and rotifers. (A) Sample large cobbles covered with phototrophic mat in a stream. (B) Scrape biofilm, wrap in aluminum, and preserve in liquid N_2. (C) Thaw biofilm, extract meiofauna, sort, clean and measure (e.g., here, nematodes and rotifers). (D) Extract pigment contents of c.300 individuals in 200-μl MeOH, and elute pigment solution in HPLC following Barlow et al. (1997). (E) Compare biomarker pigments in meiofauna versus in biofilm to infer algal diet. (F) Mount some specimens and scan under a confocal laser scanning microscope (e.g., with 488-nm laser emission and 665-nm fluorescence record to track chlorophyll). (G) Localize and measure fluorescent objects to get insight into digestion process. Chlorophyll fluorescence is in red. *White arrows*: Diatoms in rotifer gut indicating further vacuolar digestion. *Modifed from Majdi et al. (2012c), Mialet et al. (2013), more details therein.*

3. Some of the animals you observed reproduce asexually, while others have separate sexes and internal fertilization. What are the advantages of each mode of reproduction for these fauna? If a stream is prone to unpredictable, severe disturbances (e.g., floods, droughts, etc.), which reproductive mode might prevail? Why?
4. Numerous meiofauna taxa are ubiquitous in their distribution in many parts of the world. For which type of approach/assessment could ubiquity represent a crucial advantage?
5. For which type of habitat(s) are meiofauna better suited than macrofauna to be used as conventional indicators of contamination? Why?
6. A small stream is known to be receiving pollution from a copper mine. How could you use a combination of field sampling and laboratory trials to assess if this pollution is likely impacting meiofaunal populations at this site?
7. Are meiofauna just little macrofauna, or are they biologically and ecologically distinctive? Think about how body size might affect the diets, degree of feeding specialization, response to physical disturbance, and dispersal.
8. The number of temporary waterways is expected to increase with climate change and water extraction. What kinds of meiofauna might thrive better in there? What biological and ecological traits would you expect these animals to possess?

14.5 MATERIALS AND SUPPLIES

Reagents

Water (distilled or MilliQ filtered stream water, filter pore-size <5 μm preferable)
Six percent $MgCl_2$ solution (73.2 g/L)

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Ethanol (ETOH: 90% to be diluted to 40%, 50%, 60%, 70%, 80% in wash bottles)
Glycerol 10% and 4% buffered formalin (to buffer, saturate with sodium borate or hexamine)
Rose Bengal stain (add 1 g Rose Bengal powder in 1 L of 10% formalin, or 300 mg in 1 L water)
Bouin's fixative/stain (add 25 mL 37% formalin and 5 mL glacial acetic acid to 75 mL saturated solution of picric acid). The effects of the three chemicals on tissues tend to balance each other, resulting in excellent nuclear and cytoplasmic staining.
Ludox TM-50 (colloidal silica, initial density 1.4 g/cm^3, dilute to c.1.14-1.18 g/cm^3)
Methanol (MeOH: HPLC-grade for pigment extraction and elution gradient in HPLC)
Lysis buffer (EDTA 0.2 mM, NaOH 25 mm, pH 8)
Neutralizing solution (Tris−HCl 40 mm, pH 5)

Nematode Growth Medium (NGM)

1. Prepare K_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$ (1-M stock solutions), NaCl, Bacto peptone, Agar, Agarose and Cholesterol stock solution (5 mg/mL in EtOH, stored at −20°C).
2. Prepare potassium phosphate buffer for use in Step 5 by mixing 132 mL of K_2HPO_4 (1 M) with 868 mL of KH_2PO_4 (1 M). Autoclave or filter-sterilize.
3. Dissolve 3 g NaCl, 2.5 g bacto peptone and 17 g Agar in 1 L H_2O. Autoclave, then cool down the solution to 55°C.
4. Add 25 mL potassium phosphate buffer, 1 mL $MgSO_4$, 1 mL $CaCl_2$, and 1 mL Cholesterol and fill up to 1 L with sterile water.
5. Mix the solution well and pour (c.20−25 mL) to sterile Petri dishes while still hot.

Lysogeny Broth (LB-medium) for E. coli *growth*

Dissolve 0.5-g casein peptone, 0.25-g yeast extract, and 0.5-g NaCl in 50 mL water, all in a 250-mL Erlenmeyer flask. Cork with aluminum folia and autoclave.

E. coli *(strain OP50-Uracil deficient)*

Under sterile conditions, inoculate LB-medium prepared above with c.200 μL of a frozen *E. coli* pellet, and incubate for 17 h at 37°C on a shaker (200 rpm)

M9-medium

Dissolve 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl, and 250 mg $MgSO_4 \cdot 7H_2O$ in 1 L water

Field Material

Trowel or piece of plastic cut from a milk container for scooping up sediment
PVC corers (c.2−3 cm diam., 5−10 cm long; can be made from 30-mL syringe)
Brush-samplers (can be made from a 30-mL syringe; or Peters et al., 2005)
Nitex 500, 125, 40, 10-μm to build sieves and enclosures (see Section 14.2.2)
Shovel and Bou-Rouch pump (see Section 14.2.2)
Buckets, dissecting trays, wash bottles, toothbrushes, sample jars, plastic zip-lock bags, cooler

Laboratory Material

Baermann-Funnels (see Section 14.2.2), fridge and freezer (−20°C), fume-hood, drying-oven (up to 80°C)
Centrifuge and centrifuge tubes, magnetic stirrer, incubator, 1-mg precision balance
Potentially HPLC, LC-MS, and PCR with associated goods.

Microscopy Material

Stereomicroscope (c.10−50× magnification), with camera if possible
Compound microscope (up to 1000× magnification), with phase contrast if possible
Potentially Laser Scanning Spectral Confocal Microscope and associated image analysis software
Plastic Petri dishes (9 and 15-cm diam.) or other sorting trays, millimetric paper
Microscope slides, cover slips, and CMC-mounting media
Irwin loops and homemade worm pickers and mouth-pipettes (see Section 14.3.1)

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