

# Chapter 10

## Invertebrates and Plants

WENDELL R. HAAG, ROBERT J. DiSTEFANO, SIOBHAN FENNESSY,  
AND BRETT D. MARSHALL

### 10.1 INTRODUCTION

Invertebrates and plants are among the most ubiquitous and abundant macroscopic organisms in aquatic ecosystems; they dominate most habitats in both diversity and biomass and play central roles in aquatic food webs. Plants regulate and create habitats for a wide array of organisms (Cooke et al. 2005). Snail grazing and bivalve filtering profoundly alter habitats and communities (Harvey and Hill 1991; Vaughn and Hakenkamp 2001). Aquatic habitats in North America support extremely diverse floras and invertebrate faunas; groups such as crayfishes and freshwater mollusks reach their highest worldwide diversity here. Crayfishes are important economically for human food, fishing bait, and the aquarium pet trade industry (Nielsen and Orth 1988; Huner 1997). Freshwater mussels have been exploited heavily in North America since at least the mid-1800s for freshwater pearls, button production, and currently for cultured-pearl bead nuclei (Anthony and Downing 2001).

Macroinvertebrates and aquatic plants are the foci of many fisheries studies because of their importance in aquatic ecosystems, the imperiled status of many species, and the increasing presence of harmful invasive species. A disproportionate number of aquatic plants and invertebrates are imperiled relative to terrestrial species. For example, more than 50% of the 579 plant species considered of special concern in Pennsylvania are aquatic (Cronk and Fennessy 2001). Freshwater mussels and snails are among the most imperiled animals in North America (Strayer et al. 2004); about 35 mussel species and 40 snail species have become extinct in the last 50 years (Neves et al. 1997). Although only four species of crayfishes are federally listed as endangered in the USA, about half of the 363 species in the USA and Canada are considered imperiled (Taylor et al. 2007), illustrating the frequent disparity between formal conservation status and actual conservation risk. Conservation status of aquatic insects is poorly known; however, the local extinction rate of aquatic insects in Illinois exceeds that of fishes and mussels (DeWalt et al. 2005), and stoneflies may be comparable to mussels and fishes in their degree of imperilment (Master et al. 2000). Moreover, invasive aquatic plants and invertebrates have jeopardized the integrity of many aquatic ecosystems (Pimentel et al. 2000; Holeck et al. 2004).

This chapter provides an introduction to sampling and study methods for freshwater macrophytes and macroinvertebrates. The term macrophyte refers to all macroscopic aquatic vegetation and does not apply to microscopic plants such as phytoplankton and periphyton (Cooke et al. 2005). We do not cover zooplankton or phytoplankton; these organisms are treated separately in Chapter 9 because of their fundamental importance in aquatic systems and because their pelagic habits require specialized sampling approaches. Lowe and LaLiberte (2006) describe methods for sampling periphyton. We limit our coverage of macroinvertebrates to groups that are commonly

of interest in freshwater fisheries studies, such as nonplanktonic crustaceans, aquatic insects, and mollusks. Information on sampling other aquatic macroinvertebrates (e.g., sponges, annelids, nematodes, flatworms, mites, and meiofauna) can be found in Smith (2001), Thorp and Covich (2001), and Hauer and Lamberti (2006); however, many of these groups can be sampled with the methods described in this chapter. The scope of this chapter is huge even when focusing on freshwater ecosystems; therefore, we were forced to exclude marine ecosystems even though many methods used in freshwater are applicable in marine habitats. Chapters 6 and 7 describe trapping methods for commercially important marine invertebrates. We suggest the following sources as an introduction to the literature on sampling marine macroinvertebrates and plants: Caddy (1989), Raffaelli and Hawkins (1996), Jamieson and Campbell (1998), Little (2000), Eleftheriou and McIntyre (2005), McLachlan and Brown (2006), Bakus (2007), and Gray and Elliott (2009).

We focus on the following objectives common in fisheries management and research: (1) identification of organisms, (2) sampling to estimate diversity, spatial distribution, abundance, and biomass, (3) sample handling and marking of individuals, (4) quantification of life history or population parameters such as age, growth, and fecundity, and (5) use of macroinvertebrates and plants as bioindicators. We limit our discussion of life history and population parameters to decapod crustaceans (crayfishes and shrimps) and mollusks because these organisms are often the primary focus of fisheries studies. In contrast, fisheries biologists are rarely called upon to study life history parameters of aquatic insects, even though estimating insect abundance is often a primary goal in fisheries studies. Methods for life history study of other groups can be found in references cited in Table 10.1.

## 10.2 IDENTIFICATION AND TAXONOMY

Fisheries biologists must be able to identify their study animals, but the level of identification needed varies according to study goals; in many cases, higher-level identification (family or genus) may be adequate. Identification of plants and invertebrates poses a number of challenges. Resources for identifying macroinvertebrates and aquatic plants are summarized in Table 10.1.

### 10.2.1 Plants

The taxonomy and systematics of North American aquatic plants are stable for most groups. Most aquatic plants can be identified to genus or species by use of morphological characters of the stems, leaves, flowers, and fruit. Aquatic plants occur as four major growth forms (Cooke et al. 2005): (1) submersed, that is, the plant is rooted in the substrate and the entire plant is underwater (e.g., watermilfoil); 2) floating-leaved, that is, the plant is rooted but leaves float on the surface (e.g., water lily); 3) floating, that is, the entire plant is floating or suspended and not rooted in the bottom (e.g., duckweed); and 4) emergent, that is, the plant is rooted in the bottom but a portion of the plant emerges into the air (e.g., cattail). Aquatic plants generally have a single growth form, but some species can occur in more than one form (e.g., pondweed).

### 10.2.2 Crustaceans

Family and genus level classifications of most crustacean groups are stable, but species relationships remain problematic and many undescribed species exist (e.g., Smith 2001). Most crustaceans can be identified to genus at any time of the year based on morphological characters. However, species determination of some requires examination of sexually mature individuals,

**Table 10.1** Identification resources for North American freshwater macrophytes and macroinvertebrates.

| Group                                   | Region                           | References                                                                                                                        |
|-----------------------------------------|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Macrophytes                             | General                          | Fassett 1972; Prescott 1980; Cook 1990                                                                                            |
|                                         | Canada and Great Lakes           | Fink 1994; Borman et al. 1997; Newmaster et al. 1997; Curtis 1998                                                                 |
|                                         | Southeast                        | Beal 1977; Godfrey and Wooten 1979, 1981; Beal and Thieret 1986 Dressler et al. 1987; Aulbach-Smith et al. 1990; Tobe et al. 1998 |
|                                         | Midwest                          | Beal and Monson 1954; Winterringer and Lopinot 1977; Brooks and Hauser 1981                                                       |
|                                         | Northeast                        | Gleason and Cronquist 1991; Holmgren et al. 1998; Crow and Helquist 2000                                                          |
|                                         | Southwest                        | Correll and Correll 1975                                                                                                          |
|                                         | Northwest                        | Steward et al. 1963; Guard 1995                                                                                                   |
| Macroinvertebrates—<br>all groups       |                                  |                                                                                                                                   |
| Including insects                       | General                          | Thorp and Covich 2001; Voshell 2002                                                                                               |
|                                         | Northeast                        | Peckarsky et al. 1990                                                                                                             |
| Excluding insects                       | General                          | Smith 2001                                                                                                                        |
| Crustaceans                             |                                  |                                                                                                                                   |
| All groups                              | General                          | Fitzpatrick 1983                                                                                                                  |
| Crayfishes and<br>freshwater<br>shrimps | General                          | Hobbs 1976, 1989; Fetzner 2005                                                                                                    |
|                                         | Southeast                        | Hobbs 1981; Taylor and Schuster 2004                                                                                              |
|                                         | Midwest                          | Page 1985; Pflieger 1996                                                                                                          |
|                                         | Northeast                        | Crocker 1957, 1979; Meredith and Schwartz 1960; Jezerinac et al. 1995                                                             |
|                                         | Canada                           | Crocker and Barr 1968                                                                                                             |
| Amphipods and<br>isopods                | General                          | Holsinger 1972                                                                                                                    |
| Fairy shrimps                           | General                          | Belk 1975                                                                                                                         |
| Aquatic insects                         | General                          | McCafferty 1981; Merritt and Cummins 1996                                                                                         |
| Mollusks                                |                                  |                                                                                                                                   |
| All groups                              | Canada                           | Clarke 1981                                                                                                                       |
|                                         | Southwest                        | Taylor 1987; Wu 1989                                                                                                              |
| Snails                                  | General                          | Burch 1982                                                                                                                        |
|                                         | Midwest                          | Wu et al. 1997                                                                                                                    |
|                                         | Northeast                        | Jokinen 1983, 1992; Martin 1999                                                                                                   |
| Bivalves                                | General                          | Burch 1972, 1973                                                                                                                  |
|                                         | Canada and Great Lakes<br>states | Sietman 2003                                                                                                                      |
|                                         | Southeast                        | Howells et al. 1996; Parmalee and Bogan 1998; Brim Box and Williams 2000; Bogan 2002; Cicerello                                   |

**Table 10.1** Continued.

| Group | Region    | References                                                       |
|-------|-----------|------------------------------------------------------------------|
|       |           | and Schuster 2003; Bogan and Alderman 2004; Williams et al. 2008 |
|       | Midwest   | Cummings and Mayer 1992; Oesch 1995; Bleam et al. 1999           |
|       | Northeast | Strayer and Jirka 1997; Nedea et al. 2000                        |
|       | Northwest | Nedea et al. 2005                                                |

especially males, limiting identification to the reproductive season (Smith 2001). Problems with species level identification are most acute in regions with diverse faunas (e.g., crayfishes in the southeastern USA and isopods), and fisheries biologists needing species identification in these areas should seek out regional experts. In other regions, isopods, amphipods, crayfishes, and shrimps can be identified to species more easily.

**10.2.3 Aquatic Insects**

Most aquatic insects can be identified to family or genus based on readily observed morphological characters. Routine species level identification is beyond the scope of most fisheries studies because identification is based on morphology of adult male genitalia or wings (e.g., stoneflies, mayflies, and caddisflies) or microscopic examination of larval head capsules (midges). The adult stage of most aquatic insects is aerial and brief (e.g., a mayfly that lives for one year may spend only a few days as an adult), and specimens may not be obtainable during much of the year.

**10.2.4 Mollusks**

Snails can be identified to genus in most cases, but species level taxonomy of many snail groups is in disarray. Traditionally, species determinations are based on qualitative shell characters, microscopic examination of the radula (the rasping structure within the mouth), or male reproductive structures. However, because of high phenotypic plasticity of many morphological characters, molecular phylogenies show little congruence with traditional classifications (Lydeard et al. 1998; Wethington and Lydeard 2007). Similar to crayfishes, these problems are most acute in regions with diverse faunas (e.g., southeastern USA), but in other regions snails can be identified to species more readily.

Freshwater bivalves of the superfamily Unionacea (referred to in this chapter as mussels) can be identified to species throughout most of North America. Identification of mussels is usually based on subtle, qualitative shell characters and involves a gestalt that can seem distinctly unscientific at first. Consequently, dichotomous keys are rarely useful; rather, the most effective learning approach to identifying a regional fauna is to spend time with a specialist or in a comprehensive research collection. Despite the apparent ambiguity of diagnostic characters, concepts of mussel diversity and species identities are relatively stable and have been corroborated by molecular studies even though classifications at the genus level and above may require extensive revision (Campbell et al. 2005). The only region where species identifications for a significant part of the fauna may not be possible is the southern U.S. Atlantic Coast. These streams are dominated by members of the genus *Elliptio* and have highly variable shell morphologies; specialists have not reached a consensus about their identification (Bogan 2002).

Fingernail clams (family Sphaeriidae) can be identified to genus, but species level relationships are poorly understood and fewer species may exist than are currently recognized (Smith 2001). The invasive bivalves, the Asian clam and *Dreissena* spp. (e.g., the zebra and quagga mussels), can be identified readily based on shell characters.

### 10.3 SAMPLING METHODS

Most invertebrates, and especially plants, are sampled more easily than are fishes because of their lower mobility. For example, freshwater mussels and snails rarely elude their capturer after detection. On the other hand, many invertebrates are small, cryptic, or buried in the substrate, making their detection difficult, especially in turbid or deep water. Most macroinvertebrates covered in this chapter are benthic (i.e., living in association with the bottom) and can be sampled using similar methods and gear types. A common goal in fisheries studies is to sample the entire invertebrate assemblage (e.g., to estimate prey availability for fish) rather than to focus on a single taxonomic group. We therefore first describe methods that can be used to sample a wide variety of organisms simultaneously and then discuss specialized approaches necessary for specific organisms. Most organisms can be sampled in multiple ways, but the choice of a sampling method is dictated by the specific goals of the study as well as the physical conditions under which sampling will occur. Each sampling method has applications and limitations.

#### 10.3.1 Qualitative versus Quantitative Sampling

Sampling methods are usually considered either qualitative or quantitative (Table 10.2). However, that distinction is somewhat blurry because most qualitative methods can be quantified, or at least standardized, in some way. We define quantitative methods as those that yield absolute estimates of the numbers or masses of organisms in the study area in units that are not specific to the sampling method (e.g., number/m<sup>2</sup>) and that can therefore be extrapolated to estimate total population abundances or biomasses. We consider qualitative methods those that provide either unitless measures (e.g., species presence or absence) or relative measures of abundance in units that are specific to the sampling method (e.g., catch per unit effort [ $C/f$ ]) and cannot be extrapolated beyond the scale of the study.

With appropriate sample sizes and designs, quantitative methods can yield highly precise and repeatable estimates of abundance or biomass. Estimates of this type potentially allow detection of small differences in populations or assemblages across space and time. Quantitative estimates are also necessary in studies of secondary production or bioenergetics in which it is necessary to estimate the amount of energy available in a system. However, along with precision and repeatability come several disadvantages. Most quantitative methods are laborious, time-consuming, and expensive, limiting the scale at which they can be applied. Consequently, quantitative methods are often impractical when sampling over large areas and in a wide variety of habitats, such as in studies to estimate species richness or describe the distribution of a species. Furthermore, quantitative methods can be ineffective on low-density populations because too few individuals are encountered to provide useful data; such situations require a prohibitively large number of quantitative samples.

Qualitative sampling is usually easier, faster, and cheaper than quantitative sampling. It is the most effective way to estimate species richness and presence or absence (e.g., Vaughn et al. 1997). The robustness of species richness estimates and the ability to detect the presence of a species, especially if it is rare, depend on the number of individuals collected and the diversity of

**Table 10.2** Summary of sampling methods for freshwater macrophytes and macroinvertebrates; comparative ratings are low (L), medium (M), and high (H).

| Method                         | Taxonomic groups                                   | Habitats                                           | Study goal                                                                         | Effort<br>required | Repeatability | Bias |
|--------------------------------|----------------------------------------------------|----------------------------------------------------|------------------------------------------------------------------------------------|--------------------|---------------|------|
| <i>Qualitative methods</i>     |                                                    |                                                    |                                                                                    |                    |               |      |
| Hand sampling                  |                                                    |                                                    |                                                                                    |                    |               |      |
| Visual search                  | Plants, crayfishes, mollusks                       | All; clear water required                          | Species richness, species distribution, assemblage composition, relative abundance | L                  | L             | H    |
| Rake                           | Plants                                             | All                                                | Species richness, species distribution, assemblage composition                     | L                  | L             | M    |
| Tactile search                 | Mussels, large snails                              | Soft substrates                                    | Species richness, species distribution, assemblage composition, relative abundance | L                  | L             | H    |
| Nets                           | Crustaceans, insects, snails (in vegetation)       | All; restricted to shallow water                   | Species richness, species distribution, relative abundance                         | L                  | M             | M    |
| Traps                          | Crayfishes, shrimps, insects (adults), some snails | All                                                | Species distribution, relative abundance                                           | M                  | M             | H    |
| Introduced substrates          | Insects, crayfishes, some bivalves                 | All                                                | Relative abundance                                                                 | M                  | H             | H    |
| Intercept methods              | Plants                                             | All; primarily used in lakes                       | Species richness, species distribution, assemblage composition, relative abundance | M                  | M             | M    |
| Excavation of crayfish burrows | Crayfishes                                         | Floodplains                                        | Species richness, species distribution                                             | H                  | L             | H    |
| Shell collection               | Mollusks                                           | All                                                | Species richness, species distribution                                             | L                  | L             | M    |
| Brail                          | Mussels                                            | Large rivers                                       | Species richness, species distribution                                             | H                  | L             | H    |
| Skimmer dredge                 | Mussels, large snails                              | Lakes and large rivers; limited to soft substrates | Species richness, species distribution, assemblage composition, size structure     | H                  | M             | L    |

Table 10.2 Continued.

| Method                         | Taxonomic groups                                                             | Habitats                                                    | Study goal                                         | Effort required | Repeatability | Bias |
|--------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------|-----------------|---------------|------|
| <i>Quantitative methods</i>    |                                                                              |                                                             |                                                    |                 |               |      |
| Quadrat sampling               |                                                                              |                                                             |                                                    |                 |               |      |
| Visual examination             | Plants                                                                       | All; limited to clear water                                 | Abundance, biomass, size structure                 | M               | H             | L    |
| Whole substrate sample         | Mussels                                                                      | All                                                         | Abundance, biomass                                 | M               | M             | M    |
|                                | Mussels                                                                      |                                                             | Abundance, biomass, size structure                 | H               | H             | L    |
| Surber-type samplers           | Crustaceans, insects                                                         | Streams; limited to shallow areas with current <sup>a</sup> | Abundance, biomass, size structure                 | M               | M             | M    |
| Stovepipe and suction samplers | Smaller crustaceans, insects                                                 | All; difficult in cobble                                    | Abundance, biomass, size structure                 | H               | M             | L    |
| Whole substrate samplers       | Smaller crustaceans, insects, mollusks (only when present at high densities) | All; limited to gravel or finer substrates                  | Abundance, biomass, size structure                 | H               | H             | L    |
| Mark-recapture                 | Crayfishes, shrimps, mollusks                                                | All                                                         | Abundance, size structure                          | H               | M             | M    |
| Gerking-type samplers          | Smaller crustaceans, insects, snails                                         | Macrophyte beds                                             | Abundance, biomass, size structure                 | H               | M             | M    |
| Depletion sampling             | Crayfishes, shrimps                                                          | Streams                                                     | Abundance, biomass, size structure                 | H               | M             | M    |
| Remote sensing                 | Plants                                                                       | Lakes and large rivers                                      | Abundance, biomass, size structure                 | M               | H             | M    |
| Drift nets                     | Insects, mollusk larvae                                                      | Streams                                                     | Abundance, biomass                                 | M               | H             | L    |
| Plankton nets                  | Insects, mollusk larvae                                                      | Lakes and large rivers                                      | Abundance, biomass                                 | M               | H             | L    |
| Visual censuses                | Crayfishes                                                                   | All; clear water required                                   | Species richness, species distributions, abundance | M               | M             | M    |

<sup>a</sup> Fully enclosed Surber-type samplers may be used for crayfishes in lentic habitats.

habitats sampled rather than on the quantitative rigor of the method itself (Gotelli and Graves 1996; Vinson and Hawkins 1996). Qualitative methods can maximize the number of organisms sampled as well as the spatial and temporal coverage of samples. Using a variety of qualitative methods further increases the likelihood of sampling all or most species in an assemblage. The accuracy of estimates of the relative abundance of species within an assemblage is also dependent on the number of individuals sampled, and therefore qualitative sampling may also be superior to quantitative sampling for this purpose.

Qualitative methods can be quantified or standardized, yielding estimates that may be used to assess relative differences in abundance of organisms over time or among sites. However, these estimates are often greatly affected by sampling conditions and several sources of bias. Despite efforts to control confounding variables, estimates may have such high variability that they have power to detect only huge differences in abundance (Strayer and Smith 2003). Nevertheless, standardized qualitative methods are useful for initial attempts to describe spatial patterns of organisms. Qualitative samples may reveal coarse-scale patterns of distribution or habitat associations. These results can then be combined with more intensive quantitative methods to estimate abundance directly within strata previously identified by qualitative sampling. Such stratified sampling can greatly reduce variation among quantitative samples, thereby increasing precision of abundance estimates (section 10.3.3.1). Occupancy estimation models also may allow broader and more robust inferences from qualitative sampling and presence–absence data. These models estimate the probability of species occurrence in an area (e.g., a watershed or a stream reach) based on the probability of detection in qualitative surveys at representative sites (MacKenzie et al. 2006).

### 10.3.2 Qualitative Methods

#### 10.3.2.1 Hand Sampling

Large sessile or slow-moving organisms such as plants, mussels, snails, crayfishes, and large aquatic insects can be searched for visually and sampled effectively by hand or with a small dip net. Forceps may help to collect intact small specimens such as snails and aquatic insects (e.g., true bugs) that can bite. In deep water, crayfish and other mobile organisms can be collected using a diver-operated suction gun; this device is easier, faster, and less damaging to specimens than hand collecting (Davies and Ramsey 1989). The effectiveness of visual searches can be increased greatly by the use of a clear-bottomed viewing scope, snorkeling gear, scuba, or a surface air supply (hookah rig) (Figure 10.1); snorkeling is generally more effective than a viewing scope (Strayer et al. 1997), and a hookah rig is preferable to scuba in most situations because the diver is not encumbered by heavy tanks and a finite air supply. All of these gears eliminate surface glare and disturbance, and the magnifying effect of water aids greatly in detecting small specimens. Deepwater plant communities can be sampled by observing plants from a boat and gathering samples with a rake (Figure 10.2A), grapnel, hook, or dredge (Haynes 1984; Buchan and Padilla 2000). However, the efficiency of boat sampling varies with stand homogeneity and stem density (Golterman et al. 1988), and boat sampling can miss 15–50% of species observed in underwater surveys (Capers 2000).

A major problem with visual searches and hand sampling is the difficulty of finding organisms that are concealed within cover or buried in the substrate. Insects, snails, and small crustaceans (e.g., amphipods and isopods) can be collected by removing rocks, woody debris, roots, plants, or other substrates from the water and inspecting them for clinging animals. Immediately

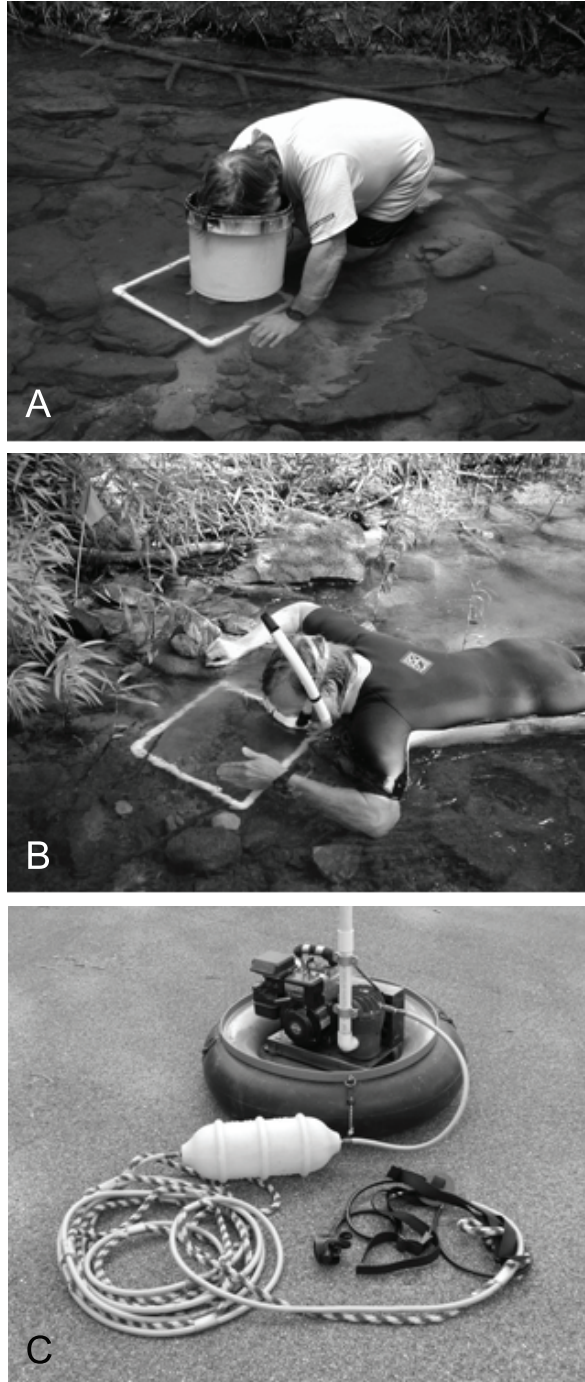


**Figure 10.1** Gears used for conducting visual searches: (A) searching a quadrat with a Plexiglas-bottomed viewing scope; (B) searching a quadrat with snorkeling gear—the biologist is fanning the substrate with his hand to remove sediment and expose partially buried organisms; and (C) surface air supply (hookah rig); the white object at center is reserve air cylinder, the regulator is at lower right, and on this model, the air compressor is mounted on an inner tube (photos by W. R. Haag).

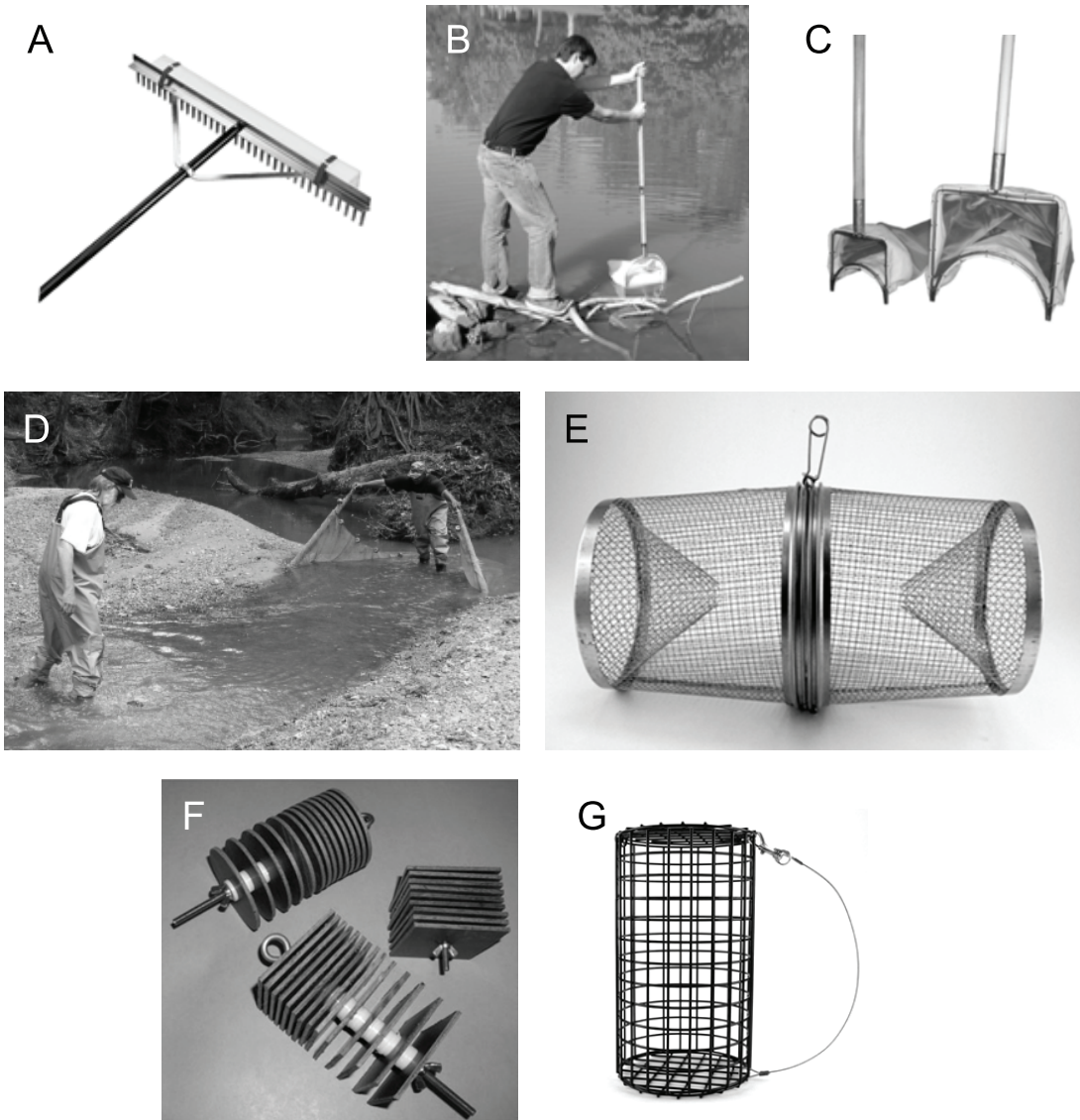


**Figure 10.2** Qualitative sampling gears for plants and invertebrates: (A) plant rake; (B) D-frame invertebrate net; (C) modified D-frame nets for collecting on woody snags; (D) seining for crayfish; (E) crayfish (minnow) trap; (F) Hester Dendy multiplate samplers; and (G) basket sampler. (Photo [D] by W. R. Haag; all other photos courtesy of Wildlife Supply Company, Yulee, Florida, with permission.)

after removing an object from the water, place it in a light-colored tray containing about 2 cm of water to facilitate inspection of the substrate; attached organisms typically soon release their hold and begin moving about the tray and can be collected with a medicine dropper or fine forceps. Aquatic insects, snails, and especially mussels may be buried partially or completely in the substrate but can be exposed by gently raking or fanning the substrate by hand (Figure 10.1B).

Undersides of nonembedded rocks and the spaces under them should be inspected carefully because many insects, crayfishes, and mollusks occur primarily in these microhabitats (Mitchell and Smock 1991; Layzer and Madison 1995).

Where sand and silt predominate (e.g., lakes and coastal plain streams), mussels and large snails are often the only hard objects in the substrate (apart from a few hickory nuts, acorns, and beer bottles) and can be sampled effectively by feeling through the substrate (tactile search); use caution to avoid injuries from broken glass and snapping turtles. Tactile searches are less effective in coarse substrates because of the difficulty of distinguishing target organisms from rocks by touch.

Hand sampling large, easily observed macroinvertebrates such as mussels (Metcalf-Smith et al. 2000; Smith et al. 2001) is most commonly quantified by timing searches to express the abundance of organisms as catch per unit effort of time ( $C/f$ ). This method is used infrequently for plants (but see Ray et al. 2001). Timed searches can be used to sample many sites relatively quickly (Strayer and Smith 2003). Densities of organisms on logs or rocks can be quantified by measuring the areas of the objects examined, but area-based quantification is difficult on other substrates.

Relative estimates of abundance made from timed searches generally suffer from several sources of bias and consequently have low repeatability. Visual searches are most effective in clear, low-water conditions; increased turbidity and depth after rainfall events or other disturbances can make visual detection of organisms nearly impossible. Even in clear water, sampling activities themselves can disturb sediments, especially silt, quickly reducing visibility to zero. The effectiveness of visual and tactile searches is highly dependent on substrate type and the amount of aquatic vegetation and other cover. The time at which sampling is conducted can have a major influence on results (section 10.3.2.8). Visual and tactile searches are strongly biased against small individuals and small species, especially during suboptimal conditions (Brown and Bowler 1977; Miller and Payne 1993; Hornbach and Deneka 1996; Rabeni et al. 1997). Furthermore, burying behavior varies among mussel species, and visual searches are biased in favor of species that do not bury deeply. Timed searches cannot generally be used to obtain reliable estimates of absolute abundance. Capture rate from timed searches may be used to predict absolute abundance, but the relationship between these two variables often has high error and does not allow precise estimation of abundance (Strayer et al. 1997; Smith et al. 2003).

#### 10.3.2.2 Nets

Nets are a standard tool for sampling a variety of macroinvertebrates (Merritt et al. 1996; Barbour et al. 1999; Moulton et al. 2002) and can collect small organisms that are easily overlooked by hand collecting alone as well as highly mobile species. Nets have the additional advantage of being relatively insensitive to variation in water clarity.

Selection of net mesh size is a primary consideration and should be determined based on the size of the target organisms. Environmental monitoring programs for insects most often use a standard mesh size of 500- $\mu$ m bar width (Carter and Resh 2001), which retains large larvae but reduces the number of early instar larvae and other small organisms that are not used in bioassessment metrics (section 10.6).

Perhaps the most widely used and effective qualitative sampling gear for insects and crustaceans is the long-handled D-frame net (Figure 10.2B); variants have rectangular, triangular, or round frames. A D-frame net can be used by one person in a variety of habitats. These nets are

either swept through the water to capture swimming organisms, held stationary on the bottom in current to capture organisms dislodged by disturbing the substrate immediately upstream (kick sampling), or swept through vegetation or soft substrates to collect snails and small bivalves (e.g., fingernail clams and *Corbicula* spp.).

The basic design of the D-frame net has been modified to collect more efficiently in specific habitats. The Needham scraper (Usinger 1956; Peterson 1959) is a coarse hardware cloth net (mesh about 1 cm<sup>2</sup>) with a steel plate along the lower edge that allows the net to be swept through fine sediments to capture large, burrowing dragonfly larvae. The Needham net (Peterson 1959) and apron net (Usinger 1956) are similar modifications designed to collect macroinvertebrates in macrophyte beds. A variant of the D-frame net has been developed to collect insect larvae from submerged large woody debris (Figure 10.2C).

In shallow water (<1 m depth), seines (Chapter 7) are effective and widely used for capturing crayfish (Stelzer and Burton 1993; Flinders and Magoulick 2005; Westhoff et al. 2006). In habitats with at least some current, crayfish and many other macroinvertebrates can be captured in large numbers by kick sampling: one or two people hold a stationary seine or D-frame net (e.g., at the downstream end of a riffle) while others vigorously disturb the upstream substrate with their feet, working downstream toward the net; organisms are dislodged and swept into the net (Figure 10.2D). Seines can also be used in areas with no current by pulling the net through the habitat; effectiveness may be improved by having other people walk ahead disturbing the substrate. Because seines have relatively large mesh sizes, they may be ineffective for sampling smaller crustaceans (e.g., isopods and amphipods) and insects.

Net sampling can be quantified by either *C/f*, usually time sampled or number of net sweeps, or by recording the area sampled. For example, a specified number of kick net samples collected with a D-frame net is commonly used to estimate diversity and relative abundance of aquatic insects quickly in rapid bioassessment protocols (Resh and Jackson 1993). However, these estimates suffer from a number of potential sources of error and bias. In the case of timed samples, observer technique and relative efficiency of nets in different habitats or conditions varies widely (Pollard 1981). Nets, especially seines, are difficult to use in deep water or habitats with numerous obstructions. In the case of area-based quantification, it is difficult to determine the size of the area sampled precisely, and escape rates can be high, resulting in underestimates of abundance (Flinders and Magoulick 2005). The effectiveness of seines can be increased by incorporating electrofishing (Chapter 8) to yield relatively accurate estimates of crayfish abundance and size structure (Westman et al. 1978; Roell and Orth 1992; Rabeni et al. 1997). However, electrofishing is biased against small individuals because they are less affected than are large individuals, may cause loss of appendages useful for identification, and may cause stunned crayfish to remain hidden in heavy cover or under cobble or boulder substrates, resulting in underestimates of abundance (Westman et al. 1978; Roell and Orth 1992).

#### 10.3.2.3 Traps and Baiting

Adult forms of aquatic insects can be captured with emergence traps or light traps (Merritt and Cummins 1996; Smock 2006). Crayfish and shrimp can be trapped using a standard minnow trap with two conical entrances (Figure 10.2E), baited and fished overnight (Somers and Stechey 1986; Townsend et al. 2002). The most common bait is cut fish, but other baits include feed grain, fish meal, dog or cat food, sweet corn, and commercial preparations (Huner and Barr 1980; Rach and Bills 1987). Burrowing crayfish can also be captured in traps (section 10.3.2.6).

Snails in the genus *Campeloma* can be attracted to fish or carrion (Dillon 2006), and Florida apple snails can be captured efficiently in dense vegetation by means of unbaited crayfish traps (Darby et al. 2001).

Baited crayfish traps have been used to estimate relative abundances in lakes and marshes (Capelli and Magnuson 1983; Somers et al. 1996; Acosta and Perry 2000), but estimates are influenced by many variables including trap entry hole size, bait type, crayfish life history, season, lunar phase, habitat characteristics, and density of fish predators (Collins et al. 1983; Somers and Stechey 1986; Stuecheli 1991; Richards et al. 1996; Moore and Grills 1999). Trap samples can be expressed as numbers of organisms captured per unit effort expended, usually trap-days (Somers et al. 1996; Hein et al. 2007; but see Somers and Green 1993). Baited traps may attract individuals from long distances and thereby overestimate true abundances, are biased toward larger sizes because of aggressive exclusion of small individuals, and may capture seasonally skewed sex ratios (Brown and Bowler 1977; Capelli and Magnuson 1983; Olsen et al. 1991). Traps have not been used widely in streams; however they produce variable results in these habitats as well (Rabeni et al. 1997; DiStefano 2000). Baited traps are therefore most useful for estimating species richness in combination with other techniques (Riggert et al. 1999). Temporal or spatial comparisons of catch rates should be made only if variables can be tightly controlled (e.g., time of year; Litvan et al. 2010) and may be most appropriate for comparing abundances of specific life history stages (e.g., adult males; Capelli and Magnuson 1983; Olsen et al. 1991).

#### 10.3.2.4 Introduced Substrates

Insect larvae can be collected passively by placing introduced substrates (also called standardized substrates or artificial substrates) into the water, retrieving them some time later (usually 4–6 weeks), and collecting organisms that have colonized the substrates (Rosenberg and Resh 1982). Any object placed in the water can serve this purpose, but standardized gears include Hester Dendy multiplate samplers (Figure 10.2F) and basket samplers (Figure 10.2G), into which rocks, sticks, or other natural substrates are placed (Merritt and Cummins 1996; Rabeni 1996). In unstable substrates, samplers can be modified to float above the bottom to avoid being buried by shifting sediments (Beckett et al. 1998). The uniformity of introduced substrates makes them easy to examine and process. Furthermore, introduced substrates facilitate sampling in alluvial streams where most insects are attached to snags, root masses, and undercut banks, all of which are difficult to sample with conventional methods. Similarly, crayfish can be sampled with bundles constructed from leafy tops of bamboo or cane, leaves, or coarse twine (Warren et al. 2009). Introduced substrates may be preferable to baited traps for estimating and monitoring crayfish relative abundance (Dorn et al. 2005).

Settlement of planktonic larvae of *Dreissena* mussels and Asian clams can be monitored using settlement plates similar to those used for insects (Haag and Garton 1992; Borcharding and Sturm 2002). Settlement plates are used widely in marine studies (e.g., McCulloch and Shanks 2003; Yan et al. 2004), but their application is limited in North America for freshwater bivalves because native species have either direct development (fingernail clams) or larvae do not settle onto hard substrates (unionids).

Introduced substrates can be replicated and repeated with great precision and are relatively insensitive to observer bias. They are therefore powerful for assessing differences in relative abundances (measured as colonization rate) among study sites or over time; furthermore, the high precision of these estimates necessitates less replication to detect significant differences than is needed

with other methods (e.g., Surber samplers; section 10.3.3.1). The primary weaknesses of introduced substrates are that they cannot be used to estimate absolute density, some species may not readily colonize the substrates, colonization rates may vary among species, and rate of settlement has a strong seasonal component that precludes comparison of temporally variable samples.

#### 10.3.2.5 Point- and Line-Intercept Methods for Plants

Plant assemblages can be characterized with point-intercept and line-intercept methods; the point-intercept method is used most frequently (Box 10.1). Samples are taken at a series of points placed randomly, in a systematic grid, or along a transect line (Madsen and Bloomfield 1993; Rogers and Owens 1995; Titus et al. 2004; Cooke et al. 2005); point locations are predetermined and can be located in the field with global positioning system (GPS) equipment and geographic information system (GIS) software (Madsen 1999; Chapter 4). At each point, plant species are recorded as present or absent. In clear water, plants can be sampled visually within some approximated area (e.g., a visualized 2-m-diameter circle) from a boat by use of a viewing scope to eliminate surface glare. Alternatively, divers can sample a known area (e.g., 1-m<sup>2</sup> quadrat) at each point (Box 10.1). Relative abundance or percent coverage of each species within the sample area can also be estimated. In turbid water, a consistent number of rake samples can be taken at each point from a boat; however, rake samples may miss small species, and it is difficult to estimate relative abundances of plant species from them. In the line-intercept method, a line of known length is stretched between two endpoints and all plants that intersect the line are recorded (Madsen 1999); this method can provide frequency of occurrence and percent cover for individual species.

Point-intercept and line-intercept methods are considered semiquantitative because they provide measures that can be readily used to test for differences in species occurrence, diversity, and relative abundance but cannot provide estimates of absolute abundance or biomass (Madsen and Bloomfield 1993). These methods can be applied quickly over large areas because they do not require counting individual stems, which is a difficult and time-consuming component of quantitative sampling (section 10.3.3.1). Intercept methods are usually superior to quantitative methods for estimating species diversity and distribution and, in some cases, can be sensitive indicators of vegetation change (Titus 1993; Madsen 1999). However, intercept methods have limitations. First, relative estimates of plant abundance or cover are made subjectively and therefore usually have poor precision and repeatability and may differ greatly among observers (Madsen 1993; Titus 1993). Second, measures of presence versus absence or relative abundance give equal weight to large and small species or individuals even though they represent very different proportions of total plant structure or biomass (Titus 1993). Third, it can be difficult to determine if a plant falls within a sample point or intersects a transect line in strong currents (Titus 1993).

#### 10.3.2.6 Sampling Methods for Burrowing Crayfishes

Burrowing crayfish are rarely present in surface waters where they can be captured by conventional methods (Williams et al. 1974; Taylor and Schuster 2004). Excavating burrows with a shovel is the traditional approach but is laborious and time-consuming, has a low success rate, and destroys the burrow complex. The success rate can be increased by noting the presence of fresh mud at the opening to identify occupied burrows (Taylor and Schuster 2004). Adult crayfish leave burrows and forage on the surface on warm, moist nights and are easily collected by hand. Surface traps can greatly increase capture efficiency (Norrocky 1984; Welch and Eversole 2006), but effectiveness is highly dependent on favorable weather conditions (Welch and Eversole 2006).

### Box 10.1 Point-Intercept Sampling for Aquatic Plants

Suppose that we are interested in sampling the aquatic plant assemblage in a 50-ha lake that is infested with introduced Eurasian watermilfoil. Treatment to control Eurasian watermilfoil is planned in the near future. Our goal is to document baseline conditions of the plant assemblage so that comparisons can be made with conditions after treatment to evaluate its effectiveness. The lake has not been surveyed for plants previously, so we decide to use a point-intercept method with sample points located in a systematic grid to ensure even coverage of the lake. We have time to sample about 50 to 75 points; sampling at 100-m intervals gives us a total of 63 sample points as arrayed on the map below.

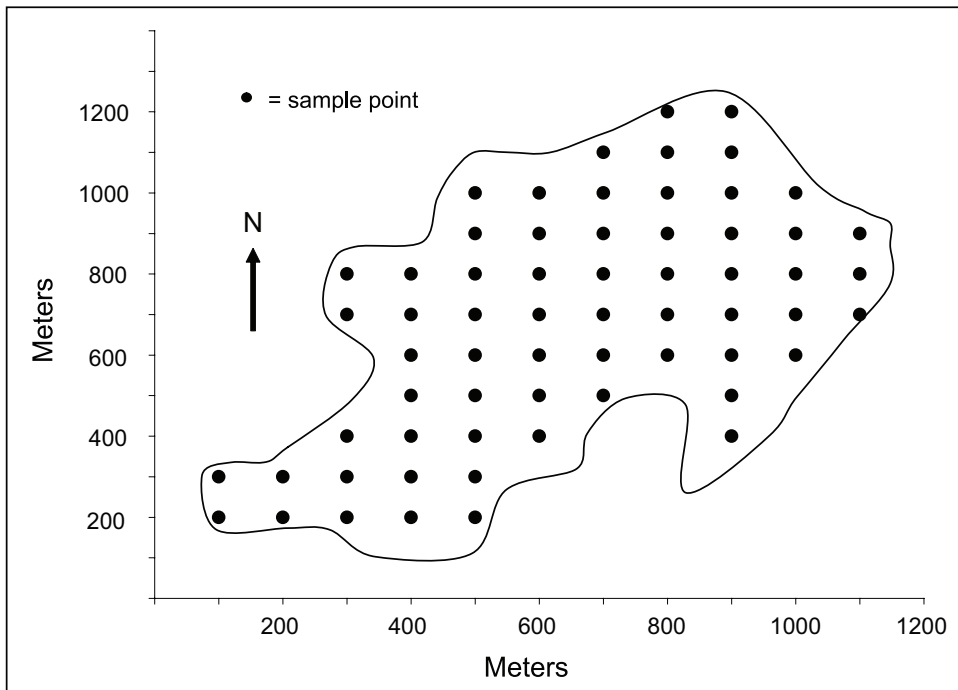


**Figure** Map of sample points.

In a boat we navigate to each point by means of a GPS unit (Madsen 1999). At each sample point, two divers descend (taking care to observe appropriate safety guidelines; Chapter 3) and place a 1-m<sup>2</sup> quadrat on the bottom. The divers record all plant species present in each quadrat and estimate the percent of the quadrat covered by each species.

Back in the office, we plot the distribution of plants in the lake. The maps below show that plants are distributed unevenly in the lake and are largely absent from the deeper, northeastern portion of the lake. Furthermore, the introduced species Eurasian watermilfoil is more widely distributed than native species such as American eelgrass.

*(Box continues)*

Box 10.1 Continued

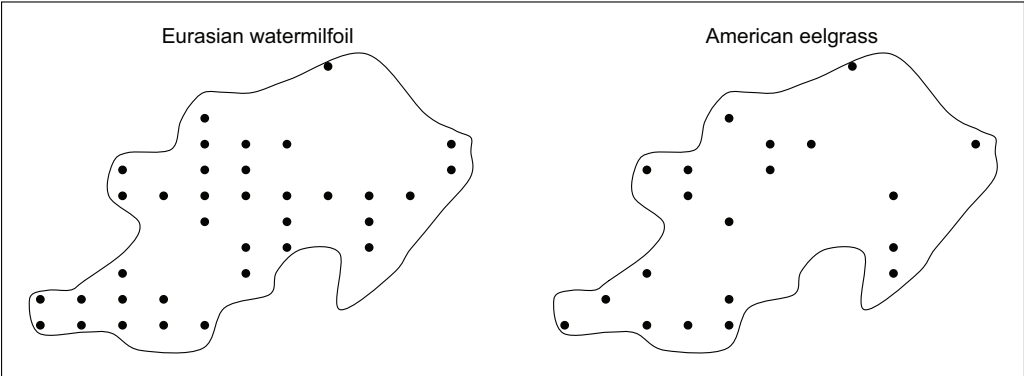


**Figure** Distribution of Eurasian watermilfoil and American eelgrass.

Next, we calculate the frequency of occurrence and average percent coverage of each species. Frequency of occurrence is simply the percentage of the total sample points ( $N = 63$ ) at which a species was present. Average percent coverage is the mean coverage of a plant species among all quadrats. Our estimates of frequency of occurrence and percent coverage also show that the plant assemblage is dominated by Eurasian milfoil.

**Table** Data on plant assemblage in sampled lake (63 quadrats sampled).

| Species               | Number of quadrats in which present | Frequency of occurrence (%) | Average present coverage (%) |
|-----------------------|-------------------------------------|-----------------------------|------------------------------|
| Eurasian watermilfoil | 35                                  | 55.6                        | 47.9                         |
| American eelgrass     | 20                                  | 31.7                        | 12.3                         |
| Canadian waterweed    | 6                                   | 9.5                         | 1.4                          |
| Pondweed              | 3                                   | 4.8                         | 0.9                          |
| Common stonewort      | 3                                   | 4.8                         | 0.2                          |

These data provide a good baseline for monitoring changes in the plant assemblage in the lake in response to control measures for Eurasian watermilfoil. The estimates of frequency of occurrence will allow us to examine changes in the distribution of Eurasian watermilfoil throughout the lake as well as any expansion by native species. In addition, because our sampling points were placed evenly across the lake, our estimates of percent coverage can be used as relative measures of the overall abundance of each species. These estimates may be useful for detecting plant assemblage responses to management if species distributions do not change but their abundances do.

#### 10.3.2.7 Other Sampling Methods for Mollusks

Because they persist after the death of the animal, mollusk shells can provide an excellent record of past and present mollusk faunas. Often, a robust estimate of species richness at a site can be obtained by searching shorelines and the streambed for empty shells, particularly when combined with data from visual or tactile searches for live animals (e.g., Smith et al. 2001; Warren and Haag 2005). When present, piles (middens) of mussel or snail shells resulting from harvest by muskrats or other predators provide a remarkable resource to the biologist; these middens are collected easily and quickly and provide an estimate of species richness and, in some cases, species relative abundance. Middens are usually found in discrete feeding stations located in cavities in the bank or under rootwads or other cover but can also be scattered along shorelines on emergent logs or large rocks. Data obtained from dead shell assemblages should be used with caution. Shells erode at different rates depending on site-specific water chemistry (Strayer and Malcom 2007); in well-buffered streams, shells persist for decades whereas in acidic streams shells can dissolve in less than a year. Furthermore, thin-shelled species erode more quickly than thick-shelled species, causing the former to be underrepresented in dead shell samples. Muskrats tend to be size selective and forage in high-density mussel beds (Hanson et al. 1989; Neves and Odum 1989); therefore middens may underrepresent large and small species and species occurring in other habitats. Moreover, the absence of shells along a shoreline does not necessarily reflect a lack of mollusks; muskrat harvest is sporadic, and few shells may be present despite the presence of dense populations just offshore.

In large rivers, mussels can be collected by brailing. A brail consists of a long pipe or board from which dangle gangs of blunt hooks. The brail is dragged along the stream bottom by a boat; when a hook contacts a live mussel, the animal quickly closes its shell on the hook, and the mussel is pulled from the substrate (Strayer and Smith 2003). Brailing can be useful for large-scale exploratory searches for mussel beds but has a low catch rate, is highly biased against some species and small size-classes, and is cumbersome to employ. A skimmer dredge pulled behind a boat can provide unbiased estimates of species richness, species relative abundance, and size structure in lakes or large rivers but is limited to sand or silt substrates with few obstructions, requires a powerful engine, and may be destructive to the habitat (Miller et al. 1989).

#### 10.3.2.8 Time of Sampling

The time of sampling can have a major influence on results. Because of differences in emergence times and longevity, sampling for plants at several different times during the year will increase chances of encountering all species present and finding flowering or fruiting specimens needed for identification (Grigal 1985; Titus 1993; Rogers and Owens 1995); however, if only one sample can be taken, it should be taken during the middle of the growing season (Adamus and Brandt 1990). Seasonal changes in aquatic vegetation biomass will also influence the effectiveness of visual searches for other organisms. Crayfish are largely nocturnal (Collins et al. 1983; France 1985), and burrowing species (section 10.3.2.6) may be absent seasonally from open-water habitats even in areas with large populations (Payne 1972). Females of some species conceal themselves when they are ovigerous (i.e., bearing eggs) or brooding young (Malley and Reynolds 1979; Corey 1988). Aquatic insects are characterized by strong seasonality in occurrence of larval stages. In headwater streams, some insect species may be abundant in early spring but absent during the remainder of the growing season; consequently, sampling for insects should ideally be conducted at several times during the year. Mussel burying behavior varies strongly by

season (Amyot and Downing 1991; Smith et al. 2001); in one population, 80% of individuals were visible at the substrate surface in spring but only 10% were visible in winter (Balfour and Smock 1995). Accounting for these temporal differences in abundance and behavior is essential to estimating species richness, presence versus absence, and species relative abundance accurately within an assemblage.

### 10.3.3 Quantitative Methods

Most quantitative sampling methods for macroinvertebrates and plants involve direct estimates of abundance obtained by sampling a known area. Indirect methods such as mark–recapture (Chapter 11) are used less frequently and mostly for large organisms such as crayfishes and mussels.

#### 10.3.3.1 Direct Estimates of Abundance

*Quadrats and transects.* The primary method used to estimate abundances of macroinvertebrates and plants is direct enumeration of all target organisms in a series of replicate samples of known area. In its most basic form, this type of sampling consists of counting organisms within a square quadrat, circular frame, or a linear transect of known width and length. Quadrats can be constructed of any strong material including rebar, PVC pipe drilled with holes to reduce buoyancy, or fiberglass rods (Figure 10.1); collapsible quadrats can be constructed using bungee cord material or flexible tubing to join the sides (Wapstra et al. 2007). Transects can be delineated by stretching a line across the sample site and sampling a prescribed distance on either side of the line or by flipping a quadrat end-over-end along the length of the transect. Transects are used primarily for larger organisms that occur at low densities (e.g., crayfishes and mussels) and are impractical for small organisms (e.g., insects and small crustaceans) and those that occur at high densities (e.g., plants). Replicated quadrat or transect samples obtained with statistically appropriate sampling designs allow precise estimation of average abundances at sites.

Submersed plant abundance is best estimated using scuba or snorkeling gear to count the number of plants in a series of replicate quadrats directly (Madsen 1993). When sampling quadrats, the number of stems of each species is counted taking care to count only plants that are rooted in the quadrat. Large quadrats or plots commonly used to sample terrestrial vegetation (Relevé or Braun-Blanquet plots, up to 100 m<sup>2</sup>) are used infrequently for aquatic plants because of the difficulty of thoroughly sampling large areas underwater. Quadrat sizes for sampling aquatic plants typically range from 0.1 to 1.0 m<sup>2</sup>, and 0.1–0.2 m<sup>2</sup> is optimal in many situations (Madsen 1993; Titus 1993). Despite advantages, quadrat sampling of plants is time-consuming and therefore restricts the scale of application. In situations where large areas must be sampled, relative measures of abundance (section 10.3.2.5) or remote sensing techniques (section 10.3.3.3) may be necessary. Direct sampling of quadrats may be difficult or undesirable under poor visibility or hazardous diving conditions. In these situations, quantitative plant samples can be collected with benthic grabs or corers; disadvantages of these samplers are (1) plant material that originates outside the quadrat may be collected, (2) large amounts of vegetation may clog the samplers, (3) they perform poorly in coarse substrates, and (4) they can sample only small areas, necessitating a large number of samples (Madsen 1993).

Quantitative sampling gear for macroinvertebrates is categorized by habitat types in which they are most effective (Table 10.2). The most common sampling device for use in streams with steady currents and rocky or gravel substrates is a quadrat or circular frame with an attached net; variants include the Surber sampler (sample area = 0.023–0.093 m<sup>2</sup>; Figure 10.3A), Hess sampler (0.1 m<sup>2</sup>; Figure 10.3B), portable invertebrate box sampler (0.1 m<sup>2</sup>), T-sampler (0.05 m<sup>2</sup>; Mer-

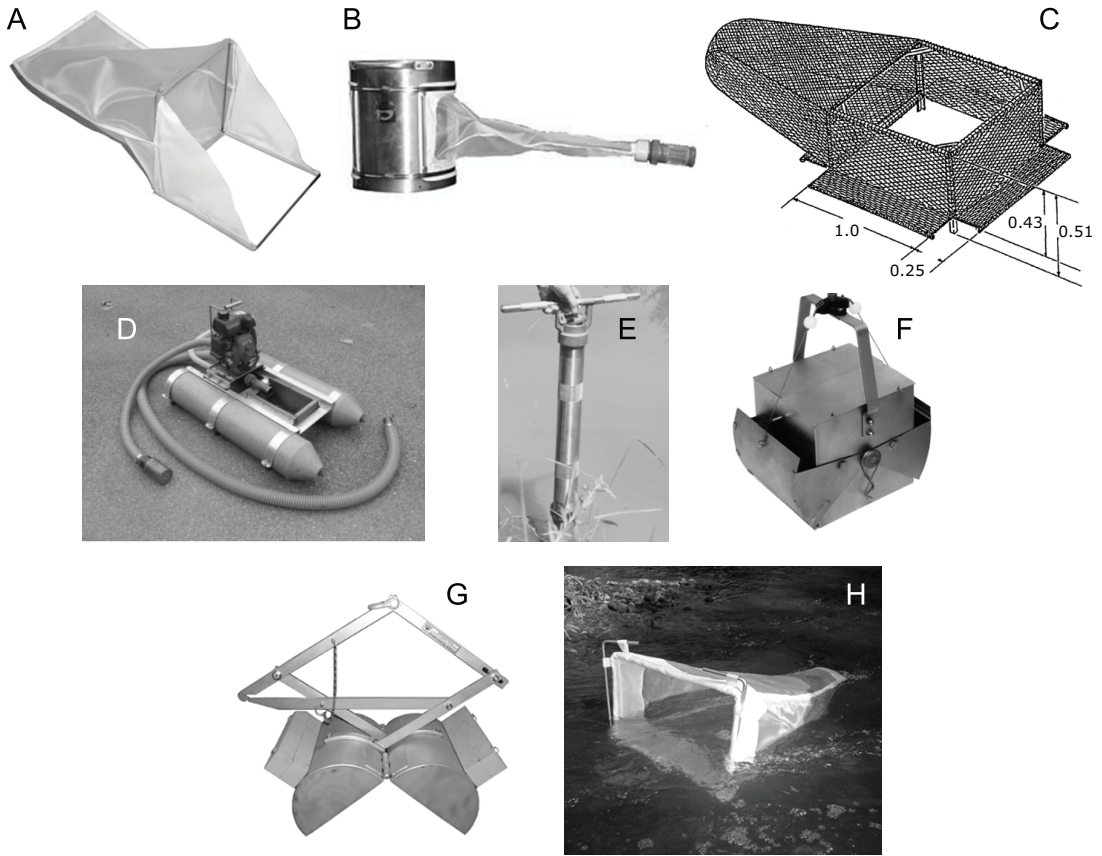


**Figure 10.3** Quantitative sampling gears for invertebrates: (A) Surber sampler; (B) Hess sampler; (C) crayfish sampler (all dimensions in meters); (D) portable suction dredge; (E) sediment corer; (F) Ekman grab; (G) Peterson grab; and (H) drift net. (Photo [D] by W. R. Haag; drawing [C] from DiStefano et al. 2003, with permission; all other illustrations courtesy of Wildlife Supply Company, Yulee, Florida, with permission.)

ritt and Cummins 1996), and the Slack sampler ( $0.25 \text{ m}^2$ ; Moulton et al. 2002). To use these samplers, the collector disturbs the substrate within the sampling frame, and dislodged organisms are washed by the current into the net. These samplers are effective for a wide range of benthic organisms including most aquatic insects and crustaceans such as amphipods and isopods. Fully enclosed samplers (e.g., Hess, T-sampler, and box sampler) reduce escapement of highly mobile organisms (Bretschko 1990). Escapement is a particular problem for crayfish, especially for larger individuals (Rabeni et al. 1997); large, modified Surber-type samplers ( $1.0 \text{ m}^2$ ) have been developed to reduce escapement and size bias and to account for the larger size and lower density of crayfishes compared with smaller macroinvertebrates (Figure 10.3C; Rabeni 1985; DiStefano et al. 2003; Larson et al. 2008). Surber-type samplers are generally ineffective for mollusks because their heavy shells prevent many or most individuals from being washed by the current into the collecting net; if these samplers are used for mollusks, the area within the quadrat frame must be carefully inspected, and animals must be placed into the net by hand.

Habitats with little or no current are difficult to sample quantitatively for invertebrates. Crayfish can be sampled by collecting or counting the number of individuals within quadrats or along transects, but escapement is a problem and must be recorded and accounted for (Lamontagne and Rasmussen 1993; Westhoff et al. 2006). Fully enclosed samplers can reduce escapement (DiStefano et al. 2003), and 1-m<sup>2</sup> throw traps (Dorn et al. 2005) can be effective for crayfish in still waters. Smaller organisms that cannot be collected easily by hand require more specialized sampling gear. Stovepipe or suction samplers can be used in gravel substrates. In shallow water, a stovepipe sampler is worked into the bottom, and then the enclosed water is pumped into a net as the substrate within the sampler is scrubbed with a brush and agitated to dislodge clinging organisms. An inexpensive stovepipe sampler can be made by removing the bottom from a steel 19-L (5-gal) stock pot or bucket (USEPA 2003). In deeper water, completely enclosed diver-operated samplers such as the dome sampler (Gale and Thompson 1975; Merritt and Cummins 1996) use motorized pumps to agitate the substrate and collect dislodged insects. It is difficult to estimate invertebrate abundances on large cobbles, boulders, and rock outcroppings. Voshell et al. (1992) developed a rock outcrop sampler similar to the stovepipe sampler but with foam packing on the bottom to seal around irregular bottom contours.

Because of their low mobility, mollusks can be sampled with quadrats or transects in any habitat in which sufficient visibility exists for their detection. Common quadrat sizes for mussels range from 0.125 to 1.0 m<sup>2</sup>, but 0.25 m<sup>2</sup> is optimal in many situations (Pooler and Smith 2005). Snails often occur in high abundances, requiring smaller quadrats and possibly subsampling with a plankton splitter (Kerans et al. 2005). The primary difficulty in estimating abundances of mollusks in quadrats or transects is detection of all individuals in the sample unit. Snails tend to cluster on the undersides of rocks, vegetation, and woody debris but can be found by careful examination of all substrate materials in the quadrat; in some cases, light excavation may be necessary to ensure that all individuals are found (Pyron and Covich 2003).

Burrowing by mussels can affect sample accuracy. Quadrat sampling using visual inspection alone leads to size and species bias similar to that of qualitative methods (sections 10.3.2.1 and 10.3.2.7). The most common solution is to excavate quadrats by digging and sifting through the substrate by hand. This requires no specialized equipment and can provide reasonably unbiased estimates of adult abundances but will result in underestimation of abundances of juveniles and other small mussels and is subject to bias because excavation techniques of collectors vary. Collector bias can be reduced by standardizing the excavation depth (usually 10–15 cm; Hornbach and Deneka 1996; Haag and Warren 1998; Obermeyer 1998). The only way to obtain unbiased samples is to remove all substrate from the sample unit and sift it through a graded series of sieves. Substrate can be removed by hand and placed in buckets or bags, but collection with a portable suction dredge (Figure 10.3D) is more accurate and efficient (Miller et al. 1997; Haag and Warren 2007). Sieving entire substrate samples from quadrats eliminates most sources of bias associated with visual searching, detects deeply buried individuals, and can provide accurate estimates of size structure and recent recruitment strength. However, sieving is time-consuming and should be used only when size bias must be eliminated (e.g., recruitment studies). Effort can be reduced by excavating only a subset of quadrats (at least 25%) and relating abundances therein to visual counts made before excavation (Smith et al. 2000; Smith et al. 2001); the proportional difference can be used to calibrate counts from the remainder of quadrats sampled using visual inspection. Routine, large-scale excavation, whether by hand or by dredge, can cause habitat disturbance in small streams, but these effects have not been evaluated widely. No short-term mussel mortality

attributable to excavation was observed in three northeastern U.S. streams (Smith et al. 2000), and no decrease in mussel abundance was observed after 10 consecutive years of annual quadrat excavation using a portable dredge at three sites in two southeastern U.S. rivers (W. R. Haag and M. L. Warren Jr., U.S. Forest Service, unpublished data).

*Whole substrate samplers.* Relatively unbiased samples of macroinvertebrates can be obtained by collecting whole substrate samples. Although mussels and snails can be collected in any substrate type with a portable dredge, this method can result in damage to more delicate invertebrates (e.g., insects and smaller crustaceans), especially in rocky substrates, and dredges are cumbersome to transport and use. In sand and silt, benthic organisms can be collected efficiently using corers (sample area < about 0.03 m<sup>2</sup>; Figure 10.3E) or Ekman (Figure 10.3F), Peterson (Figure 10.3G), or Ponar grab samplers (about 0.02–0.09 m<sup>2</sup>). Corers are usually used in shallow water and grab samplers are used from a boat in deeper water. Heavy grab samplers (Ponar samplers can weigh up to about 45 kg) are used most easily with an electric winch. Only the top 5–10 cm of substrate should be collected to avoid anaerobic sediments that slow sorting and harbor few organisms. Sediments collected by corers or grabs are sorted with sieves appropriate for the sizes of the study organisms and therefore produce highly accurate estimates of abundance and size structure. Fine gravel substrates can be sampled with some of the heavier grab samplers (e.g., Ponar), but, in general, grabs or corers perform poorly in rocky substrates.

Grab samplers can provide precise and unbiased estimates of mollusk density, especially in sand or silt substrates (Hanson et al. 1988; Kilgour and Mackie 1988; Strayer et al. 1994). Because they sample a small area (<0.1 m<sup>2</sup>), grab samplers are most practical for sampling high-density populations of small mollusks, especially Asian clams, fingernail clams, and snails. Low-density populations of unionid mussels would necessitate a large number of samples that would be laborious to obtain with a benthic grab, and large specimens might not be collected consistently by these samplers.

Quantitative sampling of macroinvertebrates in macrophyte beds and on large woody debris is challenging because quadrat samplers can be ineffective and difficult to use on such structures and large numbers of invertebrates may be associated with vegetation. If vegetation is sparse, insects and other organisms can be quantified by clipping individual plants within quadrat samples and examining them for clinging invertebrates (Keast 1984; Downing and Cyr 1985; Newman and Biesboer 2000). In dense macrophyte beds, insects, crustaceans, and snails can be sampled using a Gerking sampler or a throw trap (Chick et al. 1992; Jordan et al. 1996); both consist of a metal box that encloses a known area of the bottom (usually about 1 m<sup>2</sup>) and all associated vegetation (USEPA 2003). Material collected in the box is sieved and examined for clinging organisms. A Gerking sampler modified with a grab sampler attached to the bottom can facilitate cutting macrophytes and compartmentalizing the sediment from the macrophyte fraction of the sample (Gates et al. 1987). Abundance of insects on woody snags is best estimated by quickly cutting and collecting a section of the snag and preserving the entire sample in a bag; the snag can then be examined carefully in the laboratory, and length and average diameter of the snag can be measured to calculate surface area of the sample (Benke et al. 1984).

*Sampling design for direct, quantitative methods.* The primary challenge in using area-based samplers such as quadrats and transects is determining how many samples to take and where to take them. The number of samples needed for precise abundance estimates is a function of the size of the sampler and the density and spatial distribution of the target organisms. Ideally, one aims for a sampler size that will yield an average of five or more individuals per sample (Al-

lan 1984) and a high frequency of occurrence of the dominant species in the assemblage (Titus 1993). For sparse populations, a larger sampler will increase the number of organisms obtained per sample, thereby requiring fewer samples (Downing and Anderson 1985). However, quadrats larger than 1.0 m<sup>2</sup> are cumbersome and difficult to search effectively, and manufactured samplers (e.g., Surber and related samplers and benthic grabs) are not available in larger sizes. The number of samples necessary to obtain precise abundance estimates generally increases with decreasing density of target organisms. When organisms are sparse (usually <1 per sample) a prohibitively large sample size is required to obtain precise abundance estimates (e.g., Downing and Cyr 1985; Downing and Downing 1992). Consequently, obtaining useful abundance estimates for sparse populations or rare species is often impractical or impossible using quantitative methods (Box 10.2) and necessitates the use of qualitative approaches.

Besides sampler size and number of samples, the placement of samples is a critical concern. Most aquatic plants and macroinvertebrates (indeed, most organisms) are distributed patchily; that is, their abundance varies greatly among and within sites. Failure to account for this patchiness can result in two major problems: (1) estimates of mean abundance are not representative because samples were taken in areas of either unusually high or low abundance, and (2) the variance among samples is so great that the estimate of mean abundance has poor precision. The latter problem can occur even with large sample sizes (Box 10.2), and both problems result in estimates that allow detection of only large differences between or among areas.

If nothing is known about the spatial distribution of organisms at a site, a sampling design needs to provide representative coverage of the area. Randomly selected sample points (located by selecting random points in a coordinate grid overlaid on the site; Box 10.2) are commonly used to place quadrats or transects in a study area, but this approach can have serious shortcomings. If abundance of the target organism is high and relatively uniform throughout the study area, random sampling can provide reasonably precise estimates, but if organisms are distributed patchily, variance among samples will be high, resulting in estimates with poor precision. Prohibitively large sample sizes are necessary to overcome this problem, especially for sparse populations (Downing and Downing 1992; Strayer et al. 1997; Box 10.2). Moreover, randomly selected points are time-consuming to place and rarely give even coverage of an area unless the sample size is large (Adamus and Brandt 1990; Madsen 1993; Titus 1993), often compounding the high variance among samples or resulting in sample placement that is not representative of the site (Box 10.2).

Systematic sampling designs are usually more effective for sampling patchily distributed organisms. Systematic designs are easy to employ in the field and give even coverage of sample sites (Strayer and Smith 2003). A systematic sample consists of a series of sample points that are spaced at regular intervals (Box 10.2). Because the location of each point is dependent on the location of all others, these points are not independent and must be considered a single sample (Thompson 1992). Therefore, replicate systematic samples (usually three) are taken to estimate the variance in abundance (Strayer and Smith 2003). The primary benefit of systematic sampling is that it reduces the variance among samples caused by physical gradients or other intra-site variation that may not be of interest in the study. Removing such intra-site variation results in more precise estimates of mean abundance than would be derived from randomly selected points (Box 10.2).

Prior information about the spatial distribution of target organisms in a study area can greatly increase sampling efficiency. Variance among samples can be reduced by choosing sampling locations to account for well-known, pervasive distributional patterns of organisms. For example,

### Box 10.2 Systematic Sampling to Estimate Abundance

This example is based on a real-life example from Shades Creek near Birmingham, Alabama, which supports several snail species of conservation concern (adapted from data provided by Eric Spadgenske, U.S. Fish and Wildlife Service, Birmingham, Alabama). Habitat restoration work was proposed to benefit the snails. Prior to restoration, a baseline survey of snail populations was conducted at several sites so that effects of the project could be evaluated after completion. The study site in this example is 10 m wide by 100 m long. The schematics below are maps of the study site; the width has been stretched for visual clarity. Snails are distributed patchily within the site; the ellipse represents an area of high density and the remainder of the site has lower snail density.

We determine that we have time to sample 45 quadrats, each  $0.093 \text{ m}^2$ . Quadrats are shown laid out in a simple random design in panel (A). To accomplish this, we generate 45 pairs of random numbers with one number of each pair being between 1 and 9 (width) and the other between 1 and 100 (length); for width, we select numbers between 1 and 9 (instead of 0 to 10) to avoid selecting locations on or near the bank. After selecting these numbers, we have 45 coordinates at which we will place our quadrats. Sample size for this design is  $N = 45$  (total area sampled =  $0.093 \text{ m}^2 \times 45 = 4.185 \text{ m}^2$ ), but examination of the map shows that the coordinates we selected randomly give rather uneven coverage of the site. The high snail density area covers about 25% of the total area of the site, but only 11% of sample points (5 quadrats) fall within this region.

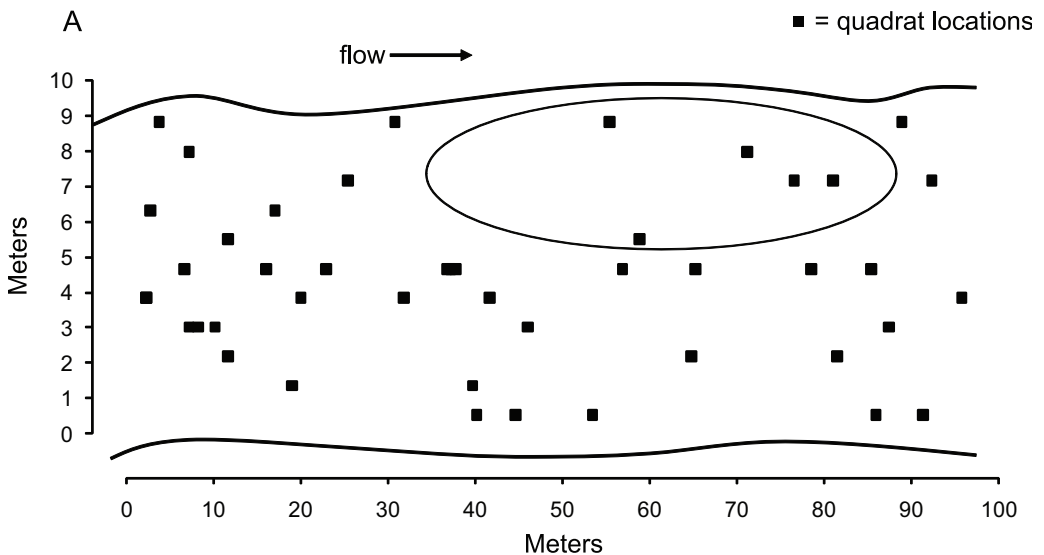


**Figure** Schematic of the study site at Shades Creek, Birmingham, Alabama; the width has been stretched for visual clarity. The ellipse represents an area of high snail density.

(Box continues)

**Box 10.2 Continued**

In panel (B), 45 quadrats are shown laid out in a systematic design consisting of three randomly positioned grids. Each grid is composed of 15 quadrats spaced regularly across the site. To give even coverage of the site, we decide that each of the three grids will be an array of three rows of quadrats across the width of the site and five rows of quadrats along the length of the site. Spacing of the quadrats within the grid is determined as width/number of quadrats ( $9/3 = 3\text{ m}$ ) and length/number of quadrats ( $100/5 = 20\text{ m}$ ). We determine the starting location for each grid randomly by selecting three pairs of numbers with one number being between 1 and 3 (width) and the other between 1 and 20 (length). Our pairs of random numbers turn out to be 1 and 6, 1 and 16, and 2 and 14; these locations are represented on panel (B) by the large symbols in the lower left corner. Each of the three different symbols represents a different grid. To determine the location of the other 14 quadrats in each grid, we add 3 m to the starting location for width and 20 m to the starting location for length and continue in this manner until all 15 quadrats have been located in each grid. The location of each quadrat in a grid is dependent on the location of all other quadrats in that grid; therefore, these quadrats are not independent, and all 15 quadrats in a grid collectively constitute one sample. Because the location of each grid was selected randomly, the grids are independent, and the sample size is  $N = 3$ ; however, the total area sampled is the same as the random design ( $4.185\text{ m}^2$ ). Each grid covers the site evenly and the percentage of quadrats that falls in the high density area (10 quadrats, 22%) is similar to the spatial coverage of the high snail density area (about 25%). Furthermore, each of the three samples has similar numbers of quadrats in the high density area (3 or 4 quadrats).

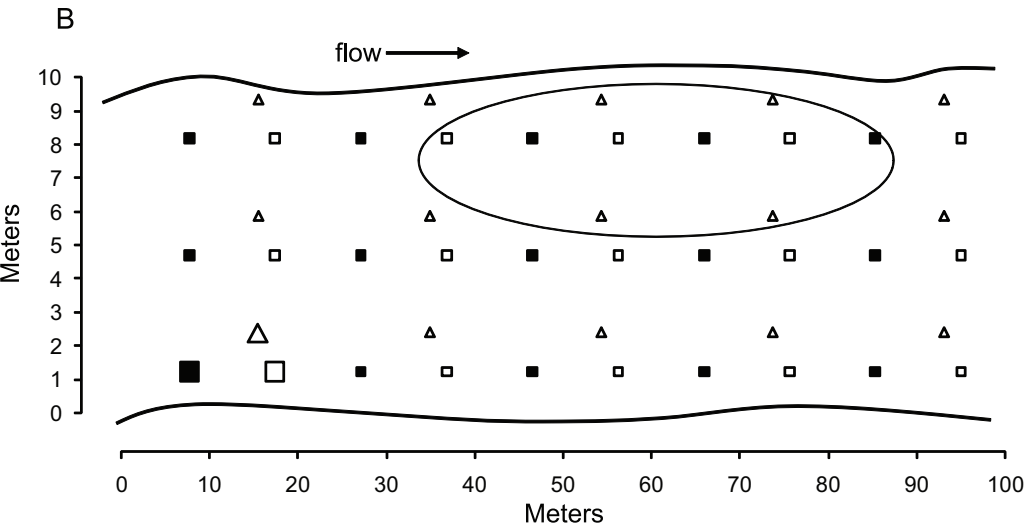


**Figure** Systematic design consisting of three randomly positioned grids; each grid is composed of 15 quadrats spaced regularly across the site. Three random starting locations are represented by the large symbols in the lower left corner; quadrats within grid represented by matching small symbol.

*(Box continues)*

**Box 10.2 Continued**

**Table** Results from the two sampling designs. Standard error (SE) is calculated as standard deviation (SD)/ $\sqrt{N}$ . Minimum detectable change is the percentage increase or decrease that could be detected with power of  $1 - \beta = 0.90$  (Zar 1996).

| Species                          | Estimated<br>mean<br>abundance<br>(per quadrat) | Standard<br>deviation | 95% confidence<br>interval | Precision<br>(SE/mean) | Minimum<br>detectable<br>change (%) |
|----------------------------------|-------------------------------------------------|-----------------------|----------------------------|------------------------|-------------------------------------|
| <i>Random design (N = 45)</i>    |                                                 |                       |                            |                        |                                     |
| <i>Elimia</i> sp.                | 63.7                                            | 60.47                 | 45.8–81.7                  | 0.141                  | 66                                  |
| <i>Leptoxis</i> sp.              | 2.5                                             | 6.32                  | 0.6–4.4                    | 0.377                  | 176                                 |
| <i>Pleurocera</i> sp.            | 0.1                                             | 0.36                  | 0.0–0.2                    | 0.537                  | 279                                 |
| <i>Systematic design (N = 3)</i> |                                                 |                       |                            |                        |                                     |
| <i>Elimia</i> sp.                | 83.7                                            | 8.31                  | 63.0–104.3                 | 0.057                  | 35                                  |
| <i>Leptoxis</i> sp.              | 3.2                                             | 0.56                  | 1.8–4.6                    | 0.101                  | 62                                  |
| <i>Pleurocera</i> sp.            | 0.1                                             | 0.08                  | –0.1–0.3                   | 0.462                  | 374                                 |

Mean abundances estimated using the two designs are fairly similar for each species, but *Elimia* abundance is lower for the random design, probably because of the disproportionately low percentage of sample points that fell in the high-density area. However, the main difference between the two designs is the precision of the estimates. The random design yields estimates for which the SD is nearly equal to or greater than the mean, resulting in precision that is much lower than that of the systematic design. Nevertheless, the precision of the estimate of *Elimia* abundance from the random design is respectable (values <0.20 are generally considered good for field studies). However, for this species we can expect to detect only a major decrease or increase in abundance (at least 66%). For the other two species, precision is low, and we have little chance of detecting anything but the most drastic changes in abundance (>>100%).

The systematic design greatly improves the precision of estimates for both *Elimia* and *Leptoxis*; SDs are much less than the mean, and precision is less than or equal to 0.1. For *Elimia*, we can expect to detect a fairly small change in abundance (at least 35%), and our ability to detect a change in *Leptoxis* abundance is much improved over the random design. For *Pleurocera*, which is rare at the site, neither design provides estimates that are useful for monitoring changes in abundance apart from detecting huge increases (>28 times the current abundance).

This example illustrates several important concepts about sampling. First, the precision of quantitative estimates is strongly dependent on the density of the organisms. It is nearly always easier to obtain precise estimates for common organisms than for rare species. Second, although larger sample sizes often improve precision, the sampling design is equally if not more important than the number of samples. Finally, the potential ability of the study to detect differences among samples statistically should be an integral part of study

**Box 10.2 Continued**

design and planning. Many monitoring and other sampling programs yield loads of data but sample sizes or designs may nevertheless be inadequate to meet the goals of the study. Careful planning before sampling is necessary to avoid this situation.

many aquatic plant assemblages show strong, predictable zonation along depth or other physical gradients. Transects or series of quadrats placed perpendicular to a physical gradient can ensure sampling in different plant assemblage zones (e.g., traversing a depth gradient perpendicular to the shoreline; Titus 1993). Similarly, assemblages of many aquatic organisms show predictable changes along stream size gradients (e.g., Vannote et al. 1980). If the goal of a study is to estimate macroinvertebrate abundance or distribution in a watershed, samples can be allocated in a stratified design based on stream size (Box 10.3). Stratified sampling is effective whenever abundances of target organisms vary predictably among strata (e.g., different habitats) but are relatively similar within strata (Keddy 1983; Morin 1985; Bornette et al. 1998; DiStefano et al. 2003, Christian and Harris 2005). Stratified sampling provides an estimate of the mean density or population abundance over the entire study site by weighting the abundance of organisms in each stratum by the proportion of the study area covered by that stratum (Box 10.3). Accounting for differences in abundance among strata can produce estimates that have better precision than estimates calculated without accounting for this source of variation (e.g., random sampling).

Two-phase sampling, which combines qualitative and quantitative methods, can be employed if no information is available about the spatial distribution of organisms in the study area or if no a priori strata can be identified (Villella and Smith 2005). In two-phase sampling, the study area is first sampled using a qualitative method that can be employed quickly and easily over a large area (e.g., timed searches or three kick net samples) to identify strata with differing abundances. Quantitative sampling (e.g., quadrats or Surber samples) can then be used to estimate abundance in each stratum; effort is often concentrated in high-abundance strata (Strayer and Smith 2003; Villella and Smith 2005). Two-phase sampling can be employed at any scale but is especially effective at the watershed scale where it would be cost prohibitive to take enough quantitative samples to account for spatial variance and produce useful estimates of abundance.

### 10.3.3.2 Indirect Estimates of Abundance

*Mark-recapture.* Mark-recapture methods (Chapter 11) have been used to estimate population abundances and survival and recruitment rates of crayfishes, shrimps, snails, and mussels (Momot and Gowing 1977; Elser et al. 1994; Rogers et al. 2001; Henry et al. 2003; Villella et al. 2004). Because mark-recapture studies require repeated sampling over time, direct estimates of abundance may be more effective and easier to obtain for many macroinvertebrates (Strayer and Smith 2003). However, mark-recapture methods are effective for estimating abundances of cave crayfishes (Hobbs 1978; Cooper and Cooper 1997) because of their low abundances and the difficulty of sampling cave habitats effectively by means of conventional methods.

*Depletion sampling.* Estimating the abundance of highly mobile invertebrates such as crayfish and shrimp can be accomplished with depletion methods commonly used for fish. Multiple-pass depletion sampling by electrofishing (Chapter 8) has been used to estimate abundances of

crayfish and shrimp (Westman et al. 1978; Eversole and Foltz 1993; Rabeni et al. 1997; Fièvet et al. 1999). Block nets may help to reduce escapement from the sample area, but shrimp may flee from the electrical field (Fièvet et al. 1999). However, accurately estimating the area sampled and ensuring that the entire area is sampled evenly and completely is difficult in depletion sampling.

#### 10.3.3.3 Specialized Methods

*Remote sensing of plants.* Direct, physical sampling is the most accurate method for sampling plant assemblages (section 10.3.3.1) but is time-consuming and can provide only limited spatial coverage. Remote sampling of plant assemblages (Chapter 4) can provide cost-effective coverage of large areas and more objective measurements than can semiquantitative methods. Remote sampling can be off-water or on-water (Sabol et al. 2002).

Off-water methods include interpretation of aerial and satellite imagery. From these images, individual plant species and boundaries between plant communities (e.g., emergent and floating) can be distinguished based on visual qualities such as shape, tone, and texture (Marshall and Lee 1994). Aerial photos and airborne videography can be used in conjunction with a GPS and GIS to provide cost-effective, large-scale mapping of the distributions of submerged vegetation over large areas and for monitoring population changes over time (Moore et al. 2000; Sprenkle et al. 2004; Everitt et al. 2005). Both Landsat Thematic Mapper and Landsat Multispectral Scanner imagery have been used successfully to distinguish cover classes of mixed communities of submerged vegetation (Ackleson and Klemas 1987). The ability to interpret plant assemblages from remote imaging is highly dependent on conditions under which images were made; accurate interpretation is difficult or impossible under conditions of water surface roughness, deep water (>2 m), and poor water clarity (Vis et al. 2003). A subsample of remotely sensed data must be corroborated by ground truthing to verify their accuracy (Sawaya et al. 2003).

On-water methods include underwater videography (Chapter 17) and hydroacoustic sensing (Chapter 13). In underwater videography, a camera is towed behind a boat and interpretation of resulting images can provide accurate estimates of submersed plant coverage and species occurrence over large areas (Norris et al. 1997). However, accuracy is dependent on water clarity and the skill of those interpreting the resulting images. Hydroacoustic sensing involves traversing sampling transects with a boat-mounted digital echo sounder that provides a two-dimensional image of objects in the water column, including plants; resulting data are spatially referenced with a GPS (Valley et al. 2005). Hydroacoustic sensing can provide rapid and highly accurate estimates of cover, height, and biomass of submerged vegetation over large areas and is independent of water clarity (Sabol et al. 2002; Winfield et al. 2007). This method can miss sparse or short vegetation, can underestimate plant height when strong currents cause plants to lay over, and is ineffective in shallow water (<1 m); physical ground-truthing should accompany this method to correct these potential errors (Sabol et al. 2002).

*Drift nets and plankton sampling.* Apart from truly planktonic organisms that can be sampled with plankton nets (Chapter 9), most freshwater macroinvertebrates are benthic; however, some benthic macroinvertebrates are found periodically in the water column. Insect larvae are frequent in stream drift (Brittain and Eikeland 1988), and aquatic insects, particularly hemipterans and coleopterans, may dominate the pelagic and littoral zones of fishless lakes. Larvae of both native and introduced bivalves occur in stream drift or in the plankton of lakes at certain times of year (Zale and Neves 1982; Haag and Garton 1992). Organisms in stream drift are sampled with drift nets staked to the bottom so that the current flows directly into the mouths of the nets

(Figure 10.3H). Drift samples are quantified by measuring the velocity of water at the mouth of the net and recording the length of time the nets are deployed (Smock 2006). The volume of water sampled is the product of velocity  $\times$  sample time  $\times$  area of the net mouth; if drift nets are deployed at depths less than the height of the net, net area should be calculated as width  $\times$  water depth. Density of drifting organisms can be expressed as either number/m<sup>3</sup> or number/L. Abundances of organisms in the drift or in the plankton typically show strong diel patterns with higher abundance of many organisms occurring at night (Smock 2006); these patterns must be considered when planning sampling and comparing samples.

*Visual censuses for crayfish.* Visual census methods (Chapter 17) can be used to estimate quantitatively crayfish abundances in clear water regardless of depth and in most habitat types if they can be identified reliably to species by sight or if species identifications are not needed (Mueller 2002; Magoulick 2004). Quantitative visual censuses are conducted by snorkeling or scuba diving along transects of specified widths and counting all individuals in the transects or by using quadrat frames as a reference (Lamontagne and Rasmussen 1993); crayfish size can be estimated using reference objects and visual calibration methods (Dolloff et al. 1996). Although visual censuses for fish can yield highly precise and accurate abundance estimates (Dolloff et al. 1996), censuses may underestimate crayfish densities and be biased toward large individuals (Rabeni et al. 1997). Bias can be reduced by sampling at night (Collins et al. 1983; France 1985) and searching under rocks to find concealed individuals (Mitchell and Smock 1991).

#### 10.3.3.4 Estimating Biomass

Estimates of biomasses of plant and invertebrate assemblages provide essential information about the amounts of energy available to support fish and other higher trophic levels in an ecosystem (Chapter 16). Biomass is a measure of the amount of living tissue in an ecosystem at any given time and is expressed as mass per unit area (e.g., g/m<sup>2</sup>). From an energetics perspective, biomass is often more useful than abundance for characterizing the amount of energy available in a system. For example, insects may be abundant at a site, but a smaller number of crayfish may represent more biomass because of their much larger size (Rabeni et al. 1995).

Biomass estimates are necessary to estimate production. Production is a measure of the rate of accumulation of biomass per unit area over time (e.g., g/m<sup>2</sup>/year). Although communities or populations with high biomasses often also have high rates of production, these two quantities can differ fundamentally. For example, a population of large, senescent mussels experiencing little recruitment could dominate the biomass but contribute little to secondary productivity and therefore be of little importance to the energy balance of the system. In contrast, an aquatic insect population with many generations per year could represent a relatively small amount of biomass, especially seasonally, but could have a high rate of production and be of central importance to energy flow.

Estimation of biomass combines measures of mean density and size structure with masses of individual organisms and can be calculated directly or indirectly (Box 10.4). The direct approach involves collecting all plants or invertebrates within some known area (e.g., quadrats or Surber samples) and weighing the organisms, usually after drying (section 10.4.2.2); biomass is the mean mass of organisms per quadrat. The direct approach is used frequently for herbaceous plants because their size is difficult to measure (Cronk and Fennessy 2001). The direct approach can be used for nearly all plants or animals but necessitates sacrificing all individuals in the sample and is time-consuming if many samples are processed.

### Box 10.3 Stratified Sampling to Sample a Watershed

In this hypothetical example, we are interested in determining the species richness and overall relative abundance of crayfishes in a fourth-order upland watershed that has not been surveyed previously. We determine that we can sample two sites per day and that at this rate time and funding will allow us to sample, in total, 20 sites.

In upland regions that have few wetlands or other natural lentic habitats, different habitat types available to aquatic organisms (including crayfishes) vary primarily in stream size. Furthermore, we know that in other streams in the region, crayfish abundance is typically higher in first-order streams, which have few predatory fish, than in larger streams. Therefore, we decide to stratify our sampling by stream order. Stratification will ensure that we take samples from as many different habitat types as possible and will provide information about assemblage differences among habitats. If we randomly chose sample sites, we might fail to sample some habitats, particularly large-stream segments that compose a small percentage of the watershed, and our data might not provide useful habitat-specific assemblage information. Moreover, stratification will account for the high variation in abundance among streams of different sizes; failure to account for this would result in abundance estimates that have low precision.

We begin by examining U.S. Geological Survey 7.5' topographic maps to determine the number of stream segments representing each stream order. From the map below, we see that our watershed contains 43 stream segments: 27 first-order, 10 second-order, 5 third-order, and 1 fourth-order. Because of the small number of third- and fourth-order segments, we decide to combine these into a single stratum.

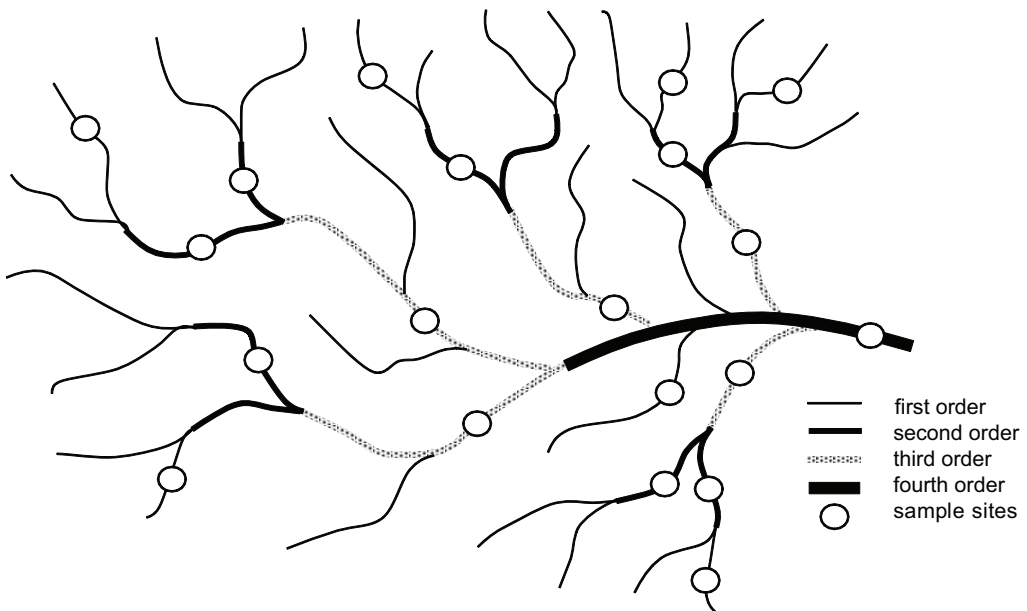


**Figure** Map of stream segments and sampling sites.

*(Box continues)*

**Box 10.3 Continued**

Most stream segments in the watershed are first-order; if we knew nothing about crayfish distribution in the area, we might decide to sample stream segments based on their relative occurrence in the watershed (see table below). However, we decide to devote disproportionately greater effort to larger streams for two reasons. First, because of the lower abundance of crayfishes in larger streams, we wish to increase our sample size there to improve the precision of our abundance estimates in this habitat. Second, because one of the goals of the study is to estimate species richness, lower crayfish abundance in large streams requires more effort to collect a sufficient number of individuals to maximize the likelihood of encountering all species in the assemblage. Therefore, we will sample all six third- and fourth-order segments, seven second-order segments, but only seven first-order segments; we randomly select second- and first-order segments for sampling. This sampling scheme provides more even coverage of sites based on stream size than if we had sampled based simply on the occurrence of each stratum in the watershed (see table).

**Table** Sampling scheme.

| Stratum<br>(order)  | Total<br>stream<br>segments<br>( $N_h$ ) | Relative<br>occurrence<br>in<br>watershed | Number of<br>samples/stratum<br>(based on<br>$n = 20$ ) | Actual<br>number of sites<br>sampled/stratum | Proportional<br>number of sites<br>sampled/stratum |
|---------------------|------------------------------------------|-------------------------------------------|---------------------------------------------------------|----------------------------------------------|----------------------------------------------------|
| First               | 27                                       | 0.63                                      | 17                                                      | 7                                            | 0.35                                               |
| Second              | 10                                       | 0.23                                      | 2                                                       | 7                                            | 0.35                                               |
| Third and<br>fourth | 6                                        | 0.14                                      | 1                                                       | 6                                            | 0.30                                               |
| Totals              | 43                                       |                                           | 20                                                      | 20                                           |                                                    |

Because estimating diversity is a primary goal of the study, we decide to use semiquantitative methods (kick sampling with a seine) to maximize the number of individuals we collect and the variety of microhabitats that we can sample at each site; this method also gives good relative abundance estimates but is biased by crayfish escapement and could be influenced by variation in sampling technique (section 10.3.2.2). At each site, we will take 20 kick net samples, each of which samples an area of 1.0 m<sup>2</sup> (Westoff et al. 2006). We go to the field and sample all 20 sites; results are presented in the table below. The table also shows the estimated mean abundance and variance per stratum and the estimate of overall crayfish abundance in the watershed. For illustrative purposes, we calculated the overall abundance based on the stratified approach but also calculated this estimate as if our 20 samples sites were taken randomly without stratification based on stream size. This unstratified estimate of the mean and its variance is simply calculated across all 20 sites, irrespective of stream size. The stratified estimate was calculated using

*(Box continues)*

### Box 10.3 Continued

$$y_{st} = \frac{1}{N} \sum_{b=1}^L N_b y_b,$$

where  $y_{st}$  is the mean abundance across strata,  $N$  = the total number of stream segments in the watershed,  $N_b$  is the total number of segments in each stratum, and  $y_b$  is the mean abundance in each stratum (Thompson 1992; McCallum 2000). The variance around this estimate is

$$\text{var}(y_{st}) = \sum_{b=1}^L \left( \frac{N_b}{N} \right)^2 \left( \frac{N_b - n_b}{N_b} \right) \frac{s_b^2}{n_b},$$

where  $s^2$  is the variance within each stratum, and  $n_b$  is the number of sites sampled in each stratum. Using the numbers in the sample table (above) and results table (below) gives us

$$y_{st} = \frac{1}{43} [27(9.5) + 10(4.8) + 6(1.5)] = 7.3;$$

and

$$\text{var}(y_{st}) = \left( \frac{27}{43} \right)^2 \left( \frac{27-7}{27} \right) \left( \frac{1.75}{7} \right) + \left( \frac{10}{43} \right)^2 \left( \frac{10-7}{10} \right) \left( \frac{3.61}{7} \right) + \left( \frac{6}{43} \right)^2 \left( \frac{6-6}{43} \right) \left( \frac{0.85}{6} \right) = 0.16.$$

**Table** Total number of crayfish collected at all sites by species and stream order.

| Species                         | Stream order |        |                  |
|---------------------------------|--------------|--------|------------------|
|                                 | First        | Second | Third and fourth |
| <i>Barbicambarus cornutus</i>   | 0            | 0      | 11               |
| <i>Cambarus cumberlandensis</i> | 0            | 2      | 31               |
| <i>C. graysoni</i>              | 0            | 0      | 3                |
| <i>C. tenebrosus</i>            | 8            | 1      | 0                |
| <i>Orconectes putnami</i>       | 366          | 120    | 62               |
| <i>O. rusticus</i>              | 954          | 550    | 76               |
| All crayfish                    | 1,328        | 673    | 183              |

(Box continues)

The indirect approach involves estimating density and size structure by means of any appropriate quantitative method and estimating the masses of individuals in the sample based on previously generated size–mass relationships (section 10.4.2.2). The indirect method is used frequently for insects, crustaceans, and mollusks (Benke et al. 1984; Strayer et al. 1994; Rabeni et al. 1995). The indirect method is advantageous for estimating biomass nondestructively and for specimens that are incomplete (e.g., invertebrates in fish stomachs); moreover, time-consuming drying and

Box 10.3 Continued

**Table** Crayfish abundance (all species, number/m<sup>2</sup>) for each site within each stream order and summary statistics for stream order and for entire watershed.

| Site and statistic     | Stream segment order |        |                  |                 |
|------------------------|----------------------|--------|------------------|-----------------|
|                        | First                | Second | Third and fourth | Whole watershed |
| 1                      | 8.3                  | 4.2    | 1.2              |                 |
| 2                      | 10.2                 | 5.2    | 2.0              |                 |
| 3                      | 9.5                  | 7.3    | 3.2              |                 |
| 4                      | 6.3                  | 3.6    | 0.8              |                 |
| 5                      | 10.9                 | 4.0    | 0.9              |                 |
| 6                      | 9.0                  | 5.6    | 1.1              |                 |
| 7                      | 12.2                 | 3.8    |                  |                 |
| Mean abundance         | 9.5                  | 4.8    | 1.5              |                 |
| Variance               | 3.61                 | 1.75   | 0.85             |                 |
| Unstratified sampling  |                      |        |                  |                 |
| Overall mean abundance |                      |        |                  | 5.5             |
| Overall variance       |                      |        |                  | 12.91           |
| Stratified sampling    |                      |        |                  |                 |
| Overall mean abundance |                      |        |                  | 7.3             |
| Overall variance       |                      |        |                  | 0.16            |

Our sampling revealed the presence of six crayfish species in the watershed. However, these species were distributed unevenly. The two species of *Orconectes* were common in all stream sizes, but *Barbicambarus cornutus* and species of *Cambarus* were rare and occurred primarily in either small or large streams. Had we sampled randomly within the watershed or proportionally with regard to stream size, we would probably have had lower sampling effort in third- and fourth-order streams and would have probably missed species such as *B. cornutus* and *C. graysoni*, resulting in a lower estimate of diversity. Our results also confirm the pattern that we see elsewhere in the region of larger streams having lower crayfish abundance than small streams.

Our estimates of relative abundance also benefited from the stratified design. The unstratified estimate of overall crayfish abundance in the watershed is considerably lower than the stratified estimate because of the inordinately large influence of low abundances in large streams. The unstratified estimate also had much higher variance because of variation in abundance related to stream size, resulting in poor precision to detect future changes in crayfish abundance or differences with other watersheds. The stratified estimate weights the abundance in each stratum by the relative proportion of each stratum across the entire watershed. This estimate (7.3/m<sup>2</sup>) is higher than the unstratified mean abundance (5.5/m<sup>2</sup>) because it takes into account the fact that most stream segments in the watershed are first-

(Box continues)

**Box 10.3 Continued**

order streams that have higher abundances than do higher-order streams. Furthermore, the variance around this estimate is much lower than it is for the unstratified mean, giving us much more power to detect differences in crayfish abundance over time or with other watersheds. Even though it is based on semiquantitative methods, this sample design provides much valuable information on this watershed, and the design is easily repeatable by other workers.

weighing of specimens is reduced or eliminated. The indirect method can also be used to estimate biomasses from previously published studies that report density and size structure of organisms if size–mass relationships exist for the study organisms.

## 10.4 SAMPLE HANDLING AND PROCESSING

### 10.4.1 Preservation

Plant specimens intended for long-term storage should be pressed and preserved using standard herbarium techniques (Haynes 1984; Stuessy and Sohmer 1996). In addition to leaves, stems, and roots (for herbaceous species), it is desirable to include flowers and fruit when possible. Some small, delicate aquatic plant species are best preserved in 50% ethanol or methanol (Haynes 1984). Rare plants can be photographed instead of collected (Fennessy et al. 2002). Plant specimens collected for biomass estimation (sections 10.3.3.4 and 10.4.2.2) should be washed to remove sediment and other extraneous matter and weighed as soon as possible after collection; if weighing is delayed, specimens should be refrigerated (Madsen 1993; Cronk and Fennessy 2001).

Selection of a short-term preservation method for invertebrates depends on study goals and the animal group. For crayfishes, four short-term (<6 months) storage methods (formalin, ethanol, isopropanol, and freezing) were equivalent for preserving initial carapace lengths and chela widths (DiStefano et al. 1994). Freshwater mussels and snails should be narcotized prior to preservation if measurements of the soft parts are needed because extreme contraction occurs in nonnarcotized specimens upon fixation (Smith 2001); narcotizing specimens is also humane and allows the fixative to penetrate tissues of bivalves and snails with opercula, which can close tightly. If bivalves are not narcotized, the shells should be kept open slightly with small wooden pegs. Specimens can be narcotized with an overdose of MS-222 (tricaine methanesulfonate) or other anesthetics (McMahon and Bogan 2001; Chapter 5). Freezing is the best method when the color of specimens needs to be maintained as all preservatives cause rapid color loss (DiStefano et al. 1994). Specimens for genetic analysis should be frozen or preserved in 95% nondenatured ethanol and never in formalin. Specimens for which accurate weights are needed should be preserved in 10% formalin; freezing and alcohol storage result in significant weight loss over time, but freezing may be acceptable if specimens are processed soon after collection (DiStefano et al. 1994; Benke et al. 1999).

Specimens intended for long-term storage have traditionally been fixed initially in 5–10% buffered formalin, 70–80% ethanol or isopropanol, or Kahle's fluid (6 parts formalin, 15 parts

ethanol or isopropanol, 1 part glacial acetic acid, and 30 parts water). Formalin is a potent carcinogen, and its use can be avoided in most cases. Formalin should always be used with proper skin protection and ventilation, preferably in a fume hood. Kahle's fluid, which includes formalin, penetrates insect tissue and preserves color better than ethanol. Larger specimens such as mollusks may need to be fixed in formalin to ensure proper preservation. Because formalin and Kahle's fluid destroy specimens over time, specimens should be soaked in several changes of water for 1–3 d after fixation and stored in 70% ethanol (Hilsenhoff 2001). Snails fixed in formalin may suffer damage to shell features, and their tissues can continue to deteriorate even after transfer to alcohol (Thompson 2004).

Most invertebrates can be fixed and stored in ethanol, thereby avoiding the health risks associated with formalin. Isopropanol may cause specimens to become brittle over time. Ethanol should be changed after initial fixation to prevent dilution, and large specimens may need to be pierced to allow entry of the fluid. Unsorted samples containing large amounts of detritus and other organic matter can be preserved whole in the field for later sorting in the laboratory, but a slightly higher concentration of fixative should be used to offset the large volumes of water in the samples, and fixative should be changed soon after returning to the laboratory. Specimens should not be stored in denatured alcohol because chemical additives used to render alcohol nonpotable cause specimens to become brittle and eventually break down; additionally, these chemicals denature DNA, rendering specimens useless for genetic analysis. Purchase of nondenatured alcohol in the USA for scientific purposes requires a permit from the federal Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF).

## 10.4.2 Size and Mass Measurements

### 10.4.2.1 Size

Consistent standard measurements of aquatic plants sizes do not exist because of their highly varied growth habits. Nevertheless, the height of aquatic plants is easily measured and provides a relative indication of the amount of biomass present as well as the amount of cover available to fish and other organisms.

The standard measurement of crayfish and shrimp size is carapace (cephalothorax) length, defined as the distance from the anterior tip of the rostrum to the posterior margin of the carapace (Hobbs 1976; Pflieger 1996; Taylor and Schuster 2004; Figure 10.4A). Sizes of isopods and amphipods are measured as total lengths or telson widths (isopods) and head lengths (amphipods) (Benke et al. 1999; Figure 10.4B). These dimensions are not typically affected by appendage loss or other common injuries or anomalies that can introduce variation into other measurements and are easily measured on live specimens. Other reported crayfish measurements include total length (anterior tip of rostrum to the posterior edge of the telson) and chela length or width (Probst et al. 1984; DiStefano 2005; Figure 10.4A). These are difficult to measure on live animals and are subject to higher variability than carapace length, but chela length or width can be useful for estimating size and mass of incomplete crayfish specimens in fish stomachs.

The standard measurement of aquatic insects is the maximum width (mayflies, caddisflies, and stoneflies) or length (midges) of the head capsule (Figure 10.4C). These dimensions are subject to little variation caused by injuries or other anomalies and, as with crayfish chelae, insect head capsules are often intact in fish stomachs, allowing size estimation of incomplete specimens. Total length is used for some groups, especially beetles, crane flies, and some hemipterans.

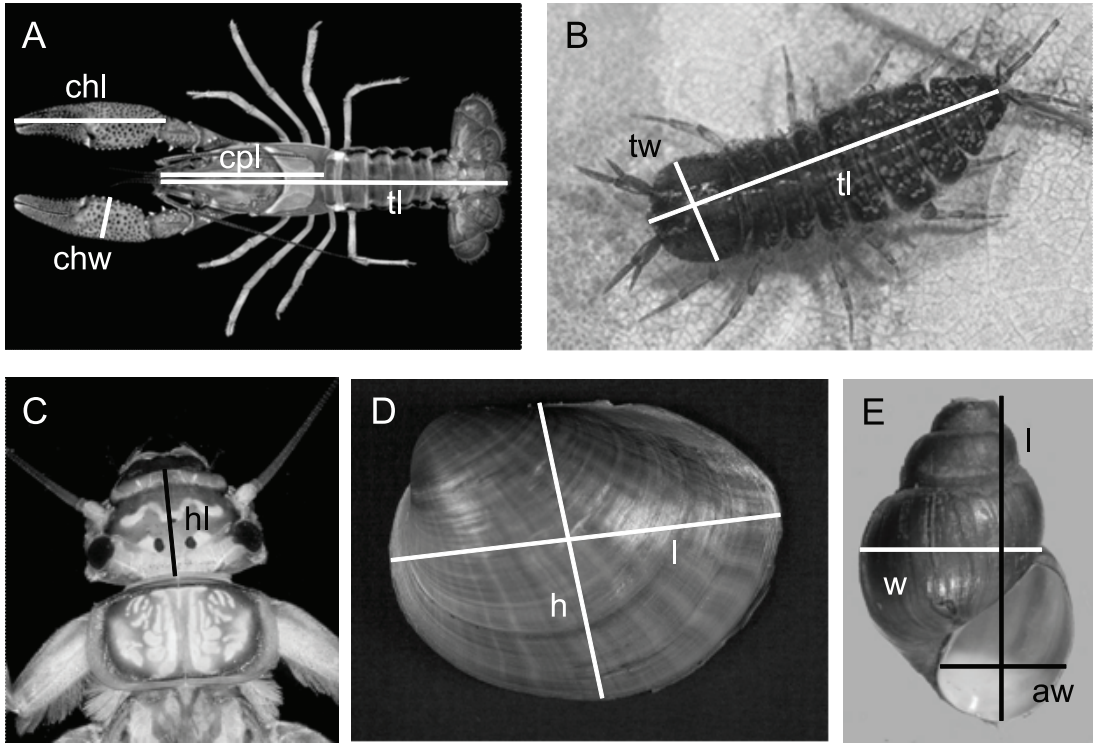


**Figure 10.4** Dimensions for measurement of representative invertebrates: (A) crayfish carapace length (cpl), total length (tl), chela length (chl), and chela width (chw); (B) isopod telson width (tw) and total length (tl); (C) stonefly head length (hl); (D) mussel length (l) and height (h); (E) snail length (l), width (w), and aperture width (aw). (Photos [A] and [C] by G. Schuster, Eastern Kentucky University, with permission; photo [B] by M. Higgins, courtesy of the North American Benthological Society ([www.benthos.org](http://www.benthos.org)), with permission; photo [D] by W. R. Haag; photo [E] by R. Dillon, College of Charleston, with permission.)

Mollusks are measured by determining shell dimensions. Shell size is highly correlated with age and tissue mass (Benke et al. 1999; Haag and Rypel 2011) and is measured much more easily than any feature of the soft tissue. Mussel size is most commonly expressed as length, measured as the longest anterior–posterior dimension (Figure 10.4D); this dimension represents the axis of greatest growth, thereby providing better measurement precision than other dimensions, and is minimally affected by shell erosion in older specimens. Snail length is defined as the distance from the apex to the lower margin of the aperture (Burch 1982; Brown 2001; Figure 10.4E), but because the apex is often eroded, shell width or aperture width (Figure 10.4E) can provide a less variable index of size (Hury et al. 1994; Johnson and Brown 1997).

#### 10.4.2.2 Mass

Plants and macroinvertebrates can be weighed either wet or dry. Wet mass is easy to measure but is subject to high variability because of the varied amounts of water retained on specimens; this is a particular problem for plants (Madsen 1993). Variability caused by residual water can be reduced by spinning plants for 15 s in a commercial salad spinner or the spin cycle of a clothes

washer (Madsen 1993; Newman and Biesboer 2000) or by blotting macroinvertebrates with paper towels. Less variable estimates of mass are obtained by weighing specimens after drying at 60–105°C for up to 48 h (depending on the size of specimens) to a constant weight (Madsen 1993; Benke et al. 1999); if weighing must be delayed, specimens can be held in a dessicator (Benke et al. 1999). The best estimates of mass are obtained by first measuring dry mass, then ashing specimens at 500°C in a muffle furnace for up to 48 h. Ashing burns off all organic matter, leaving behind only inorganic material (ash); ash-free dry mass is then calculated as dry mass minus ash mass (Chapter 16). The use of ash-free dry mass ensures that only organic material (biomass) is considered and that estimates of mass are not confounded by silt or other inorganic materials that may be in guts or on the external surfaces of specimens (Benke et al. 1999).

Most plants and macroinvertebrates are weighed whole. For plants, the most comprehensive estimates of biomass would include roots as well as stems and leaves; however, in practice most estimates of plant biomass do not include roots but only parts above the substrate surface (Madsen 1993). Mollusk shells are composed largely of inorganic material and constitute a large proportion of total individual mass; therefore, inorganic shell mass is typically not included in mass estimates. Whole snails can be decalcified in weak acid (e.g., 3% HNO<sub>3</sub>; Huryn et al. 1994) to remove most inorganic shell material; resulting mass estimates include only soft tissues and the organic fraction of the shells (Hall et al. 2006). Alternatively, soft tissues can be removed and weighed separately after gently cracking the shells; the mass of organic material in the shells and opercula can then be determined separately after decalcification (Huryn et al. 1994). Bivalve soft tissues can be easily dissected from shells after severing the adductor muscles between the shell valves. For most mussel species, mass of soft tissues and the organic fraction of shells must be determined separately because their large shells must be crushed and ground to facilitate decalcification (in 0.1 N HCl; Cameron et al. 1979) within a reasonable time period. Mollusk shells can also be ashed to estimate ash-free dry mass, but amounts of organic matter may be overestimated because of losses of substantial amounts of carbon contained in the shells as CaCO<sub>3</sub> (Cameron et al. 1979).

Masses of organisms can also be determined volumetrically or by buoyant weight; both of these methods can be used nondestructively. In the volumetric method, specimens are placed into a graduated cylinder containing a premeasured volume of water and the displacement of the specimens is recorded. The measured volume is then converted to mass by assuming a constant specific gravity of specimens (for insects, slightly >1; Cummins and Merritt 2006) or by using previously generated volume-to-mass relationships (see below) or conversion factors (Ahlgren and Bowen 1992). Buoyant masses of live bivalves (and potentially other organisms) can be determined by weighing specimens in water (Molina et al. 2005). This method reduces or eliminates variability introduced by residual water present on live specimens, and tissue and shell dry masses can be estimated based on previously determined relationships between these variables and buoyant mass.

Strong relationships exist between sizes and masses of most organisms. Regression equations describing these relationships can be used to estimate masses indirectly (but usually fairly precisely) from sizes, which are easily and quickly measured (Box 10.4). Estimating mass indirectly avoids time-consuming drying and weighing of specimens and the need to sacrifice them. This approach is dependent on having a previously generated length-to-mass equation for the study species. However, published length-to-mass equations exist for many aquatic macroinvertebrates, and family or genus level equations may be acceptable substitutions in cases in which equations do not exist for the study species (Benke et al. 1999). If no suitable equation exists, one can be

#### Box 10.4 Estimation of Biomass

This example is based on a survey of freshwater mussels in Lower Lake, an impounded section of the Little Tallahatchie River, Mississippi. Data are adapted from Haag and Warren (2007) and unpublished data (W. R. Haag). Although it is impounded, a portion of Lower Lake retains riverine characteristics and supports high mussel densities. Within this riverine area, we wish to estimate mussel density and biomass to compare how these two measures differ in their depiction of the mussel assemblage. Divers sampled six 2.5-m<sup>2</sup> quadrats within the riverine portion of Lower Lake; this quadrat size is larger than typically used for freshwater mussels (section 10.3.3.1) but was used to minimize the time necessary to reposition the boat and deploy divers.

In this example, we will compare methods for calculating biomass based on the direct and indirect methods (section 10.4.2.2). In the direct method, we dissected and weighed all mussels collected in the quadrats (ash-free dry mass). In the indirect method, we estimated mass of each individual with previously generated length–mass equations. Length–mass equations were available for all species collected in the quadrats (W. R. Haag, unpublished data) except for *Lampsilis cardium*, *Leptodea fragilis*, and *Plectomerus dombeyanus*. For *Lampsilis cardium* and *Leptodea fragilis*, we used existing equations for the congeneric species *Lampsilis ornata* (W. R. Haag, unpublished data) and *Leptodea ochracea* (Cameron et al. 1979), respectively. We estimated mass of *Plectomerus dombeyanus* by means of the length–mass equation for *Amblema plicata*, which has a similar shell form and shape. For each specimen, we estimated its ash-free dry mass ( $M$ ) by inputting length ( $L$ ) into the equation

$$M = aL^b$$

for the appropriate species, where  $a$  and  $b$  are fitted constants. Equations for selected species follow.

|                                                          |                          |
|----------------------------------------------------------|--------------------------|
| <i>Amblema plicata</i> and <i>Plectomerus dombeyanus</i> | $M = 0.0000068L^{2.938}$ |
| <i>Obliquaria reflexa</i>                                | $M = 0.0000017L^{3.333}$ |
| <i>Quadrula pustulosa</i>                                | $M = 0.0000042L^{3.215}$ |

Estimated masses from the first quadrat using the length–mass equations are tabulated below. Total estimated mass is the sum of individual masses for all specimens of each species.

**Table** Sample freshwater mussel data from quadrat one of six quadrats in Lower Lake. The measured mass is ash-free dry mass; estimated mass was generated with length–mass equations.

| Species                       | Number | Lengths (mm)                                             | Total measured mass (g) | Total estimated mass (g) |
|-------------------------------|--------|----------------------------------------------------------|-------------------------|--------------------------|
| <i>Amblema plicata</i>        | 1      | 29.4                                                     | 0.22                    | 0.14                     |
| <i>Obliquaria reflexa</i>     | 2      | 14.2, 59.6                                               | 1.80                    | 1.44                     |
| <i>Plectomerus dombeyanus</i> | 1      | 26.5                                                     | 0.14                    | 0.10                     |
| <i>Quadrula pustulosa</i>     | 10     | 14.2, 14.5, 6.7, 21.8, 41.2, 1.7, 42.1, 44.4, 46.4, 47.3 | 4.68                    | 4.96                     |

(Box continues)

**Box 10.4 Continued**

We likewise calculated estimated masses of all specimens in the data set. In large data sets with many individual specimens, calculation can be speeded up by grouping specimens into length-classes (e.g., 2-mm intervals), calculating the mass of an individual with length equal to the midpoint of the length interval, and multiplying this value by the number of individuals in that length-class. A summary of mean density and mass in all six quadrats is tabulated below. Because our quadrat size was 2.5 m<sup>2</sup>, the last line of the table shows values expressed per square meter, a more commonly used unit.

**Table** Mean density and mass of freshwater mussels in all six quadrats of Lower Lake. Totals are given per quadrat size (2.5 m<sup>2</sup>) and in a standard unit (1.0 m<sup>2</sup>).

| Species                       | Mean<br>number/quadrat | Mean measured<br>mass/quadrat (g) | Mean estimated<br>mass/quadrat (g) |
|-------------------------------|------------------------|-----------------------------------|------------------------------------|
| <i>Amblema plicata</i>        | 1.7                    | 1.54                              | 1.71                               |
| <i>Lampsilis cardium</i>      | 0.2                    | 1.06                              | 0.91                               |
| <i>Lampsilis teres</i>        | 1.0                    | 2.63                              | 2.95                               |
| <i>Leptodea fragilis</i>      | 0.8                    | 3.23                              | 2.81                               |
| <i>Obliquaria reflexa</i>     | 4.0                    | 4.06                              | 3.46                               |
| <i>Plectomerus dombeyanus</i> | 1.3                    | 6.21                              | 6.23                               |
| <i>Potamilus purpuratus</i>   | 1.2                    | 9.50                              | 9.92                               |
| <i>Quadrula pustulosa</i>     | 15.8                   | 20.04                             | 21.54                              |
| <i>Tritogonia verrucosa</i>   | 0.5                    | 2.46                              | 2.60                               |
| Total/2.5 m <sup>2</sup>      | 26.5                   | 50.73                             | 52.14                              |
| Total/1.0 m <sup>2</sup>      | 10.6                   | 20.29                             | 20.85                              |

The measured and estimated masses are similar, illustrating the accuracy of estimating mass from length–mass equations even for species for which we used equations for similar species. Moreover, comparison of density and biomass shows that these two measures provide different portrayals of this assemblage. The pie graphs below show the relative proportion of species by number and by biomass.

(Box continues)

derived from a subsample of individuals and used to estimate the masses of remaining individuals. Length-to-mass equations should be derived using a size range of specimens that is representative of the population.

**10.4.3 Tagging and Marking**

Mollusks can be individually marked using numbered tags and adhesives (Amyot and Downing 1991; Rogers et al. 2001; Pyron and Covich 2003); affixing flexible polyethylene shellfish tags with common cyanoacrylate glue is the easiest and most effective method (Lemarié et al. 2000). Coded wire tags (Chapter 11) can be inserted into the hinge ligament of mussel shells when exter-

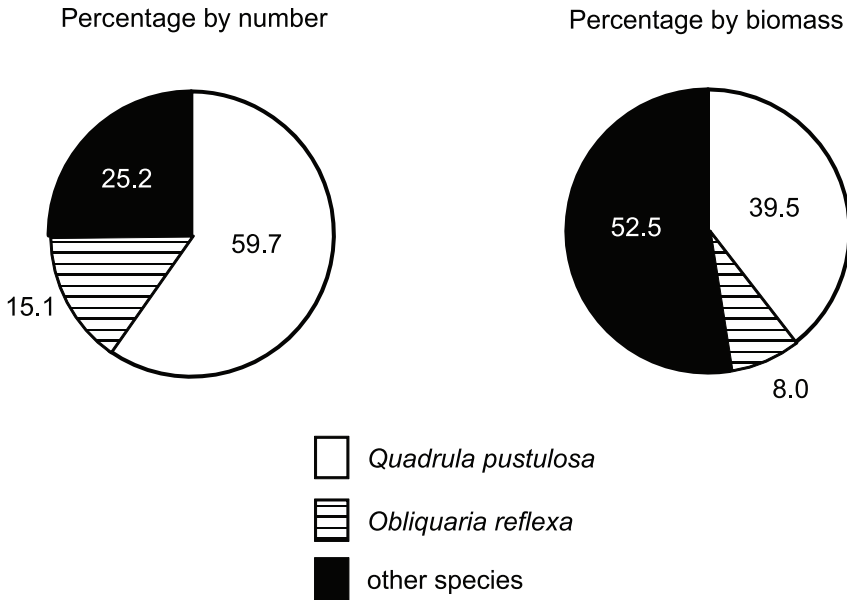
**Box 10.4 Continued**

**Figure** Relative proportion of species by number and by biomass. Species present at densities less than 4.0/quadrat were combined into one category (other species).

The assemblage is dominated numerically by *Obliquaria reflexa* and *Quadrula pustulosa*. However, because these species are relatively small-bodied compared with other species in the assemblage, they represent a much lower proportion of total biomass. The other species are mostly large-bodied, and although they make up only 25% of the assemblage numerically, they make up over 50% of the biomass in the assemblage.

nally visible tags are undesirable (e.g., to detect illegally harvested mussels; Layzer and Heinricher 2004). Tags are not easily attached to small specimens (<15 mm; but see Freilich 1989). Mollusks can be batch marked by filing notches or grooves in their shells without causing appreciable mortality (Berg et al. 1995). Large mussels have been followed using radio telemetry (Chapter 18) with mixed success (Neves et al. 1989). Passive integrated transponder (PIT) tags (Chapter 11) offer great possibilities for marking mollusks, but detection range may be limited to within 0.5 m of the antenna (Kurth et al. 2007).

Animals that periodically molt their exoskeletons are difficult to mark permanently. Crustaceans and large insects can be individually marked with numbered tags (Freilich 1989; Hagler and Jackson 2001) or batch marked by clipping or drilling holes in carapaces, branding with a soldering iron, or by painting or dyeing (Momot and Gowing 1977; Gherardi 2002; Hagler and Jackson 2001). However, all of these marks fade over time and are lost during molts. Large numbers of insect larvae can be batch marked in streams by introducing a stable isotope tracer that labels detrital food resources and ultimately the insect larvae that consume them (Payne and

Dunley 2002; Briers et al. 2004; Macneale et al. 2004). Long-term marks (batch and individual) for crustaceans include radioactive tags (Merkle 1969), internally injected ink (Hobbs 1978), external nylon suture tags (Paret et al. 1987), implanted microchips (Guan and Wiles 1997), coded wire tags (Isely and Eversole 1998), radio telemetry transmitters (Bohl 1999; Gherardi and Barbaresi 2000), visible implant fluorescent elastomer tags (Parkyn et al. 2002), and PIT tags (Bubb et al. 2002).

#### **10.4.4 Transport, Release, and Survival**

Invertebrates can quickly deplete dissolved oxygen in a small volume of water unless it is aerated vigorously. Decapod crustaceans and mollusks can be held with minimal mortality for processing or transport by keeping them cool and moist but not immersed in water (Waller et al. 1995; Bartsch et al. 2000). Most organisms can simply be released after measuring or tagging into appropriate habitats in the study area. In hard or compacted substrates, mussels should be placed with their anterior end down into the substrate; they can burrow into soft substrates without assistance. Removing mussels from the substrate and handling them causes deposition of a growth check in the shell (Negus 1966; Haag and Commens-Carson 2008), but survival rates of mussels marked with numbered tags or by shell notching are generally high (Berg et al. 1995). Currently, animal care and use protocols (Chapter 5) are not required for studying invertebrates in the USA, but this may change in the future. Regardless, biologists should minimize stress of specimens and treat them humanely.

### **10.5 LIFE HISTORY AND POPULATION PARAMETERS OF CRAYFISHES AND MOLLUSKS**

Management of any fishery requires life history information and estimates of population parameters such as age, growth, and fecundity. Measurement of these variables for crayfishes and mollusks is becoming more common because these organisms are increasingly the foci of management efforts related to commercial or recreational harvest, conservation of imperiled species, and control of invasive organisms.

#### **10.5.1 Age and Growth**

##### **10.5.1.1 Crayfishes**

Age determination of crayfish is difficult because of frequent molting and the lack of persistent hard structures. Size-frequency histograms are commonly used to assess population size or age structures (Flint 1975; Corey 1988; Parkyn et al. 2002), but older cohorts are difficult to discriminate because of reduced growth rates and subsequent size convergence (France et al. 1991). Size-frequency age estimates may be validated by mark-recapture or laboratory molt and growth observations (France et al. 1991; Reynolds 2002). Lipofuscin concentration in the olfactory lobe may provide more accurate age estimates of freshwater crayfish than size-based techniques (Belchier et al. 1998).

##### **10.5.1.2 Mollusks**

As in fishes and marine bivalves, freshwater mussels in temperate latitudes cease growing in winter and thereby produce annuli in their shells that can be used to determine ages (Chapter 15). Annuli are visible in the shell both on the external surface and in the interior of the shell in cross section (Figure 10.5). External annuli can be reliable and easily interpreted in some specimens,

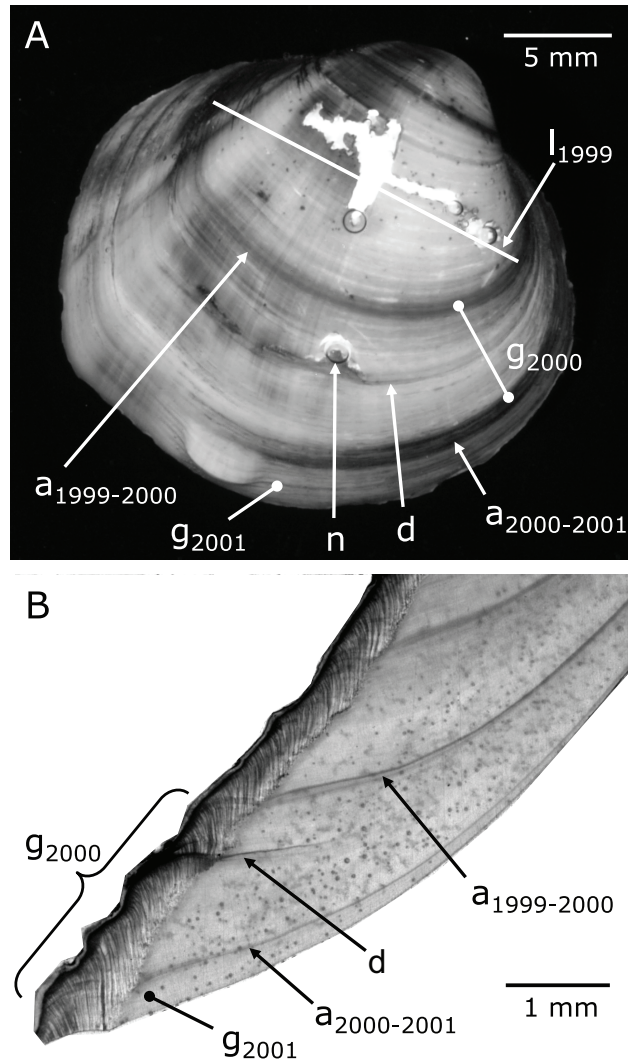


**Figure 10.5** Age and disturbance rings in mussel shells: (A) external shell rings and (B) radial shell thin section showing internal shell rings where  $a_x$  is the annulus produced in the winter of years  $x$ ,  $g_x$  is growth in year  $x$ ,  $d$  is the disturbance ring caused by notching the shell margin to produce the reference mark  $n$  for validation of annual rings, and  $l_{1999}$  is the back-measured length at the end of the 1999 growing season. Both specimens were collected in 2001 (adapted from Haag and Commens-Carson 2008, with permission.)

especially in younger individuals. However, external annuli can be ambiguous in some species, obscured by shell erosion, or too crowded in individuals of medium or advanced age to interpret reliably; consequently, ages based on external annuli are usually underestimates (Jones and Neves 2002). The most reliable and unambiguous age estimates are obtained by examining radial thin sections of shells under low-power magnification (Neves and Moyer 1988; Haag and Commens-Carson 2008). Annuli can also be resolved in the hinge ligament that joins the two shell valves

(Hastie et al. 2000), but this structure is used less often than the shells themselves and does not enable direct back-measurement of the growth history of an individual (see below). Shell rings can also be used to age freshwater snails (Shigemiya and Kato 2001) but have not been used to age North American species.

In addition to annuli, mussels produce disturbance rings in response to handling or natural disturbance, diffuse dark bands that potentially reflect periods of slower growth within the growing season, and daily rings (Dunca and Mutvei 2001; Haag and Commens-Carson 2008). Annuli can be differentiated from other rings by means of qualitative characteristics (Neves and Moyer 1988; Haag and Commens-Carson 2008). In some cases, annuli may be difficult to distinguish from disturbance rings, but this difficulty can be lessened with prior validation studies and quality control procedures (see below).

Annual ring production has been validated for only a small percentage of North American mussel species. However, in species that have been studied, production of annual rings is supported by mark–recapture studies (Neves and Moyer 1988; Haag and Commens-Carson 2008), cross dating and correlation with annual hydrologic variables (Rypel et al. 2008; Black et al. 2010), and seasonal patterns of shell chemistry (Nyström et al. 1995; Veinott and Cornett 1996; Siegele et al. 2001). Nevertheless, as for fishes, efforts to estimate mussel ages using shell rings should be preceded by validation experiments to test the periodicity of ring formation in the study population and to aid in distinguishing annuli from disturbance rings and other nonannual shell features.

Putative shell annuli can be validated by collecting and marking individuals at the middle of the growing season, returning them to the substrate, and retrieving them one year later; if the rings are annuli, one additional shell ring will be visible (Figure 10.5A). Reference marks can be made by filing a shallow notch in the shell margin (Neves and Moyer 1988), or the disturbance ring caused by initial handling can be used as a reference mark (Haag and Commens-Carson 2008). These mechanical marks work well but can be difficult to interpret in older specimens with extremely low growth rates. Exposing animals to chemical growth markers that are incorporated into the shell can provide more precise and unambiguous reference marks (Kaehler and McQuaid 1999; Fujikura et al. 2003). Annual production of shell rings can also be evaluated by analyzing putative annual patterns in shell growth. Because there is high synchronicity in growth among individuals within a population and growth is negatively correlated to annual variation in streamflow, correlations of growth among individuals and with streamflow can provide support for annual formation of rings (Rypel et al. 2008; Black et al. 2010). Deviations from these relationships can also be used as a quality control measure for interpretations of previously validated rings (Rypel et al. 2008).

Mollusk age and growth relationships can be derived from shell ring data or mark–recapture studies (Stiven and Walton 1967; Hury et al. 1994; Morris and Corkum 1999). However, mark–recapture of mussels can result in underestimates of growth because of handling disturbance during marking and subsequent recaptures and can produce strongly biased results unless marked individuals represent the full size range of individuals in the population (Haag and Commens-Carson 2008; Haag 2009). Age and growth of mussels is therefore best estimated from validated shell rings. This approach has the additional advantage of allowing reconstruction of all or a portion of the growth history of an individual. Unlike fish, for which individual growth history is estimated by inference (i.e., back-calculation; Chapter 15), lifetime growth of an individual mussel can be measured directly on its shell surface (Figure 10.5A); the location of annuli on the external shell can be determined by juxtaposing the sectioned shell with the corresponding

thin section and transferring the marks onto the shell surface with a fine-point pen (Haag and Commens-Carson 2008).

Size-frequency distributions of snails and young mussels can be used to evaluate cohort strength (Payne and Miller 2000; Pyron and Covich 2003). For mussels, the decline in growth rate with increasing age coupled with individual variability in growth result in wide overlap in length ranges of cohorts of older animals, making these cohorts difficult to differentiate. Growth of early life stages can be studied by rearing snails in the laboratory, but captive growth rates may not be representative of growth rates in the wild (Henry et al. 2003). Techniques for rearing juvenile mussels are improving (Barnhart 2006), but how well captive growth rates reflect growth in the wild is unknown.

### 10.5.2 Fecundity and Reproductive Traits

#### 10.5.2.1 Crayfishes

North American crayfishes exhibit strong sexual dimorphism and most specimens, including immature individuals, can be sexed easily, typically without magnification (Figure 10.6A–D). In males, the first two pairs of pleopods on the ventral surface of the abdomen near the base of the tail are enlarged and highly modified for sperm transfer. In females, the first two pleopods are either unmodified, vestigial, or absent, and a calcified, elliptical structure, the annulus ventralis, is present on the ventral surface of the thorax between the fourth and fifth walking legs (Figure 10.6A); the annulus ventralis is absent in males. Less easily seen sexual characters are the gonopores, which are located at the base of the fifth pair of walking legs in males but on the third pair in females (Holdich 2002; Taylor and Schuster 2004).

Female crayfish brood their eggs externally on the underside of the abdomen (Figure 10.6C); females in this condition are termed ovigerous or “in berry” (Smith 2001). Newly hatched instars are carried for 1–3 weeks after hatching. Timing of reproduction is highly variable among species and latitudes, but females are generally ovigerous between March and June (Cambaridae) or March and September (Astacidae) in the northern hemisphere (Hobbs 2001); fecundity can be determined directly by counting all eggs or hatchlings and ranges from less than 100 to 700 (Smith 2001).

#### 10.5.2.2 Mollusks

Most North American unionid mussel species are dioecious, and about 40% of species can be sexed based on external shell characters. In these species, the posteroventral margin of the female shell is inflated and expanded, presumably to accommodate the gills, which become greatly distended when gravid (Smith 2001; Figure 10.6E and F). Shell sexual dimorphism is expressed only after sexual maturity, restricting its use to individuals greater than about 3–5 years of age (Haag and Staton 2003), and is largely restricted to the tribe Lampsilini (Campbell et al. 2005). Sex and maturity can be determined by examining a small plug of tissue cut from the dorsal region of the visceral mass. Samples from this region nearly always contain gonadal tissue, and spherical oocytes can be readily distinguished from creamy-white testes by means of a dissecting microscope; immature specimens will have undifferentiated tissue that appears clear and granular (Bauer 1987; Haag and Staton 2003). Sex can be determined nondestructively by examination of gonadal fluid under low magnification (40×) collected using a biopsy needle (Saha and Layzer 2008). Some species may be difficult to sex with either method when active gametogenesis is not occurring, necessitating histological examination of gonadal tissue under higher magnification

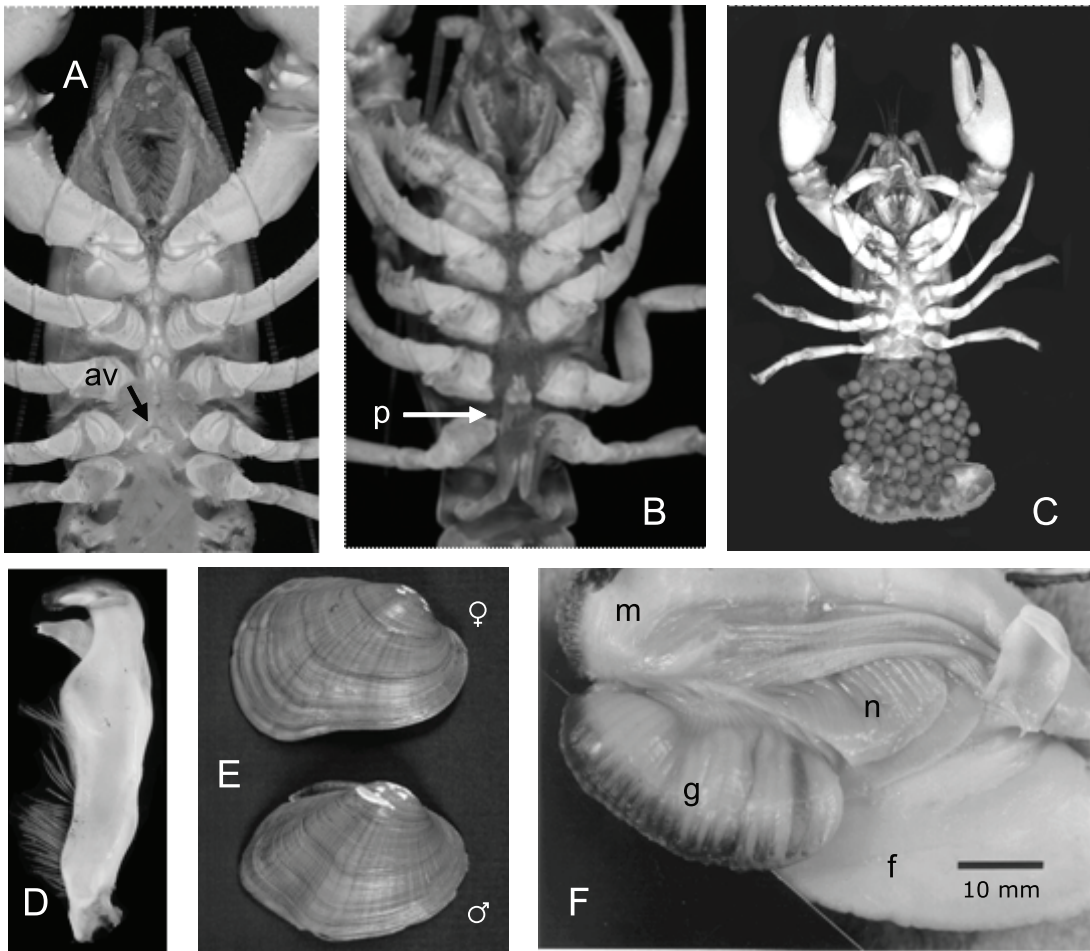


**Figure 10.6** Sexual characteristics of crayfishes and mussels: (A) female crayfish showing annulus ventralis (av) and unmodified pleopods; (B) male crayfish showing modified pleopods (p); (C) ovigerous female crayfish showing eggs attached to underside of abdomen; (D) detail of modified male crayfish pleopod (*Cambarus*); (E) shell sexual dimorphism in mussels (female top, male bottom); and (F) gravid female mussel showing the gravid portion of outer gill (g), nongravid portion of outer gill (n), nongravid inner gill (visible below outer gill), foot (f) and mantle (m). (Photos of crayfish by G. Schuster, Eastern Kentucky University, with permission; photos of mussels by W. R. Haag.)

(Saha and Layzer 2008). Histological examination is also necessary to quantify the occurrence of hermaphroditism, which is normally rare but can be substantial in some species (Downing et al. 1989).

All freshwater pulmonate snails and the prosobranch family Valvatidae are hermaphroditic; all other North American prosobranch snails are dioecious (Smith 2001). Dioecious species do not exhibit shell sexual dimorphism, and determination of sex requires examination of the tentacles, genital pore, or male copulatory structures (Brown 2001; Smith 2001) or cracking the shell to examine the gonads (Dillon 2000); both techniques require low-power magnification.

The ovary of freshwater mollusks is not a discrete structure but is insinuated with the digestive gland, making it nearly impossible to estimate fecundity by dissecting eggs from the ovary. Snail fecundity can be measured by bringing animals into the laboratory or into field enclosures, where they usually lay eggs readily; clutch size can be counted directly, but snails may lay multiple clutches over an extended period (Wethington and Dillon 1997; Hall et al. 2006; Norton and Bronson 2006). Fecundity of bivalves that release planktonic larvae (Asian clams and zebra mussels) can be estimated by maintaining animals in the laboratory and collecting released veligers (Aldridge and McMahon 1978). Unionid mussels produce larvae (glochidia) that are brooded in the female gills and then released to become parasitic on fish. Gravidity of most species can be assessed by gently prying apart the valves and peering into the shell; gravid gills are usually visibly distended (Smith 2001; McLain and Ross 2005; Figure 10.6F), but this swelling may be inconspicuous in species with low fecundity (Haag and Staton 2003). Fecundity of unionid mussels can be estimated by dissecting gills of gravid females and counting replicate subsamples of glochidia or eggs (Haag and Staton 2003). Nondestructive estimates of fecundity can be made by collecting glochidia in one of two ways. Some species release all of the glochidia in their gills soon after being collected; contents of the gills of species that do not exhibit this trait can be flushed with a hypodermic syringe (Jones et al. 2004). Evidence suggests that most mussel species in temperate latitudes produce a single clutch per year (Haag and Staton 2003), but this has not yet been tested directly.

### 10.5.3 Mussel Host Fish Identification

The host fish species required for development of the parasitic glochidia of unionid mussels is often of interest to fisheries biologists. Host fish use varies widely among mussel species; species may be generalists that use many unrelated fish species or may be specialists that can use only one or a few closely related fishes. Hosts of many mussel species are now known (Watters and Cummings 2006), but those of many others remain unknown; some host relationships reported in older literature are circumstantial or potentially erroneous and should be evaluated carefully. Because host use is often similar among closely related species, useful inferences about poorly known species can be made based on information about congeners (Haag and Warren 2003). Host use can be determined directly by exposing mature glochidia to a wide variety of potential host fishes and monitoring infections in the laboratory. Nonhost species will reject glochidia within 2–5 d, but potential hosts will carry glochidia for more than 7 d and ultimately produce active juvenile mussels (Zale and Neves 1982). Inferences about host use can also be made by examining natural infections on wild fishes (Neves and Widlak 1988), but infection rates on wild fish are often low, and attached glochidia may ultimately be rejected. Furthermore, glochidia of many species are similar and their identification in diverse assemblages can be problematic. Discriminant analysis of glochidial measurements can facilitate identification of some species, but others cannot be identified with certainty (Kennedy and Haag 2005). Molecular methods can be used to identify glochidia (White et al. 1996), but this method has not yet been used widely.

## 10.6 USE OF PLANTS AND INVERTEBRATES AS BIOINDICATORS

Bioassessment methods evaluate the overall biological integrity or “health” of a water body or site by comparing observed attributes of the biological community with those of reference sites that are assumed to be minimally affected by human activities. Reference conditions must be calibrated regionally to account for inherent differences in biological communities not related

to anthropomorphic disturbance. Bioassessment methods offer direct and often highly precise measures of environmental quality and are easier and often more informative than are studies of chemical contaminants or changes in physical habitat. Environmental degradation is rarely the result of a single pollutant or change in a physical characteristic of a water body; rather, it is usually the result of complex interactions of multiple effects. Bioassessment methods directly measure the responses of aquatic biota to these effects (Simon 2003).

The first widely used bioassessment method was the index of biotic integrity (IBI), which used fish assemblage attributes to assess stream health (Karr 1981; Fausch et al. 1984). Bioassessment methods now depend heavily on aquatic insects because of their high diversity, widespread occurrence, and relative ease of collection. Aquatic plants are valuable and sensitive bioindicators in wetlands, lakes, and some large rivers (Nichols 1998; Nichols et al. 2000; Fennessy et al. 2002; Hatzenbeler et al. 2004), but their use is limited in other habitats where they are rare or absent (e.g., rocky headwater streams or large turbid rivers), and plants can be insensitive to some compounds such as insecticides. The rapid bioassessment protocol (RBP) also provides a standardized methodology for conducting rapid and inexpensive bioassessments based on data on fishes, macroinvertebrates, and periphyton (Plafkin et al. 1989). These methods have been evaluated and refined extensively and adapted to many regions (Barbour et al. 1992; Barbour et al. 1999). Most U.S. states now have their own bioassessment protocols based on variants of the IBI and the RBP, but most are still being developed and refined (Davis et al. 1996; Barbour et al. 1999).

Most bioassessment methods used in the USA include multimetric indices. Rather than analyzing the abundances of individual taxa, multimetric indices employ a suite of biological metrics that each represents an ecological summary measure of some type (Rosenberg and Resh 1993; Lydy et al. 2000; Box 10.5). Biological metrics can be grouped into three broad categories (Table 10.3). Community stress metrics are based on the representation of specific taxonomic groups in the sample and can reflect declining ecosystem integrity depending on how those groups typically respond to disturbance (Hilsenhoff 1987; Lenat 1993). For example, the most commonly used invertebrate community stress metric is the EPT metric, which considers the abundance or species richness of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) in the sample (Lenat and Penrose 1996; Table 10.3). Because these insects are generally sensitive to stream degradation (e.g., urbanization, increased acidity, and changes in thermal regime; Resh and Jackson 1993; Wang and Lyons 2003), a decline in this metric is expected to reflect an overall decline in ecosystem integrity. In contrast, many midges (Chironomidae) are tolerant of stress, and the abundance of midges is expected to be high in a degraded system (Table 10.3).

Ecological community metrics include general ecological measurements such as species richness, diversity, and total abundance (Table 10.3). These metrics are often less sensitive than community stress metrics because low to moderate impairment may result in an increase in abundance or richness of some invertebrate groups. Community function metrics measure the relative abundance of functional feeding groups (groups of species that feed in similar ways; Table 10.3). These metrics also can be insensitive to perturbation in some cases (Karr and Chu 1999) but can respond strongly to severe degradation (Marshall 2001).

The other major bioassessment method is the multivariate approach, which uses statistical methods (e.g., discriminant functions and ordination) to evaluate simultaneously the abundances of individual taxa at a study site and compare them to abundances expected in reference conditions. The most widely used multivariate approach is the RIVPACS, or river invertebrate prediction and classification system (Wright et al. 1993, 2000; Van Sickle et al. 2006). The RIVPACS

**Box 10.5 Evaluation of Stream Health by means of a Multimetric Bioassessment Index**

A stream's "health" can be assessed by comparing its insect assemblage to that of reference streams in the region that are considered healthy. Reference conditions vary widely among regions; this example is based on reference conditions in montane regions of western Wyoming and metrics used in the Wyoming stream invertebrate index (WSII; Jessup and Stribling 2002). Eight Surber samples are taken at a site and combined into a single composite sample. After sorting the sample in the laboratory and identifying the insects, we can compute the metrics listed in the table below. Metrics 1, 2, and 3 are simply the numbers of different taxa of each of three important insect groups; this is not species richness because most insects will be identified to only genus. Metrics 4 and 5 are the percentages of the total sample represented by each of two insect orders, excluding one family in each order known to be tolerant of stream degradation. Metric 6 (dominance) is the percentage of the sample represented by the 5 most abundant taxa. Metric 7 is the percentage of the sample made up of insects that feed by scraping. The Hilsenhoff biotic index (HBI; metric 8) integrates information about tolerance of specific taxa to disturbance (Hilsenhoff 1987). Metric 9 is the BCI CTQ (biological condition index community tolerance quotient; Winget and Magnum 1979), which integrates information about tolerances of specific taxa to physical and chemical conditions at the site.

After calculating the site score for each metric, we compare these values to reference conditions by computing the metric score. Metric scores are computed using scoring criteria; for most metrics in this example this involves dividing the observed metric value ( $X$ ) by the reference value and multiplying by 100 to give a percentage. Note that score calculations for dominance, HBI, and BCI CTQ differ slightly from the others because scores of these indices usually increase with decreasing stream health (Table 10.3). After calculating all the metric scores, we compute a mean score for the site.

**Table** Metrics computed to evaluate stream health (see box text for explanation of metrics). The observed metric value ( $X$ ) is compared with metric values for a healthy stream (column "score calculation") to derive the metric score.

| Metric                                      | Calculated<br>metric value<br>( $X$ ) | Score calculation               | Metric<br>score |
|---------------------------------------------|---------------------------------------|---------------------------------|-----------------|
| 1. Ephemeroptera (number of taxa)           | 7                                     | $X/11 \times 100$               | 63.6            |
| 2. Plecoptera (number of taxa)              | 2                                     | $X/8 \times 100$                | 25.0            |
| 3. Trichoptera (number of taxa)             | 3                                     | $X/11 \times 100$               | 27.3            |
| 4. % Ephemeroptera (excluding Baetidae)     | 25                                    | $X/54 \times 100$               | 46.3            |
| 5. % Trichoptera (excluding Hydropsychidae) | 25                                    | $X/46.6 \times 100$             | 53.6            |
| 6. Dominance (% top five taxa)              | 80                                    | $[(95 - X)/(44.3)] \times 100$  | 33.9            |
| 7. % scrapers                               | 18                                    | $X/54.5 \times 100$             | 33.0            |
| 8. HBI                                      | 3                                     | $[(10 - X)/(8.6)] \times 100$   | 81.4            |
| 9. BCI CTQ                                  | 76                                    | $[(110 - X)/(65.9)] \times 100$ | 51.6            |
| Mean metric score for site                  |                                       |                                 | 46.2            |

(Box continues)

**Box 10.5 Continued**

We can evaluate the results of our assessment using the graph below, which shows each metric score and the mean score in the context of regional reference conditions. Although the HBI (metric 8) was indicative of very good conditions, most other metrics were much lower, and the mean score indicates that the overall health of our study stream is fair.

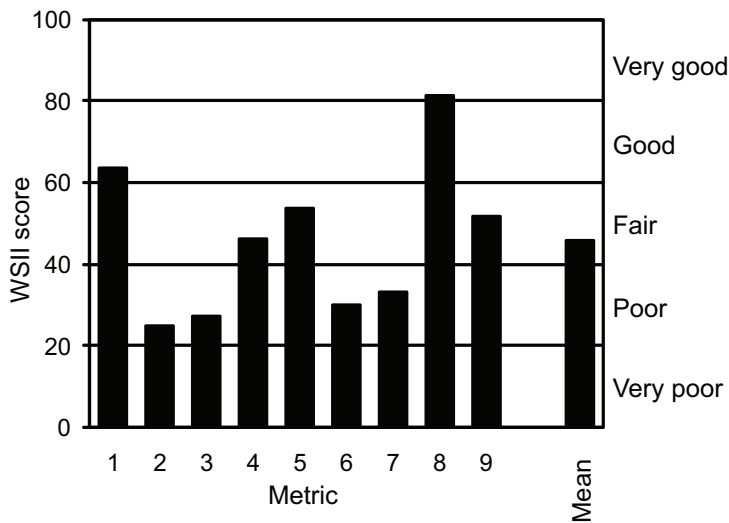


**Figure** Wyoming stream invertebrate index (WSII) scores for assessment stream in context of regional reference conditions.

uses physiochemical data from a sampling site to generate predictions about the expected fauna in the absence of environmental stress; predictions are based on physiochemical and faunal characteristics of high-quality reference reaches. The health of the site is then evaluated by calculating the ecological quality index, which is the ratio of observed to expected numbers of taxa; a high value of the index, indicating similarity between observed and expected richness, indicates good ecological health. The RIVPACS was developed in Europe and Australia and is beginning to be applied in the USA (Hawkins et al. 2000b); the U.S. states of Maine and Montana use bioassessment methods employing both multimetric indices and multivariate approaches (Barbour et al. 1999). Multivariate methods are conceptually similar to multimetric methods in their comparison of observed faunal characteristics with those of reference sites. However, the relative merits of these bioassessment methods are debated, and no preferred method has emerged (Zamora-Muñoz and Alba-Tercedor 1996; Hawkins et al. 2000a; Cao and Hawkins 2005).

A wide variety of other bioassessment methods exist that are specific to a certain group of organisms or type of habitat. The absence or decline in abundance of crayfishes has been used to assess ecosystem degradation caused by agricultural and residential run-off (Sylvestre et al. 2002) and heavy metals (Allert et al. 2009), but other crayfish species may be relatively tolerant of environmental stressors (Berrill et al. 1985). However, little is known about the sensitivity of most crayfish species, and the potential for high inter-species variability in stress tolerance restricts the widespread use

**Table 10.3** Example metrics used in bioassessment and their expected responses to stream degradation. Metrics include the EPT, which refers to the insect orders Ephemeroptera, Plecoptera, and Trichoptera; the Hilsenhoff biotic index (HBI; Hilsenhoff 1987); and the biological condition index community tolerance quotient (BCI CTQ; Winget and Magnum 1979). See Box 10.5 for further explanation of these indices.

| Metric                       | Response to degradation           |
|------------------------------|-----------------------------------|
| Community stress metrics     |                                   |
| EPT richness                 | Decrease                          |
| EPT abundance                | Decrease                          |
| Midge abundance              | Increase                          |
| Non-insect abundance         | Increase                          |
| Non-insect richness          | Increase                          |
| HBI                          | Increase                          |
| North Carolina Biotic Index  | Increase                          |
| BCI CTQ                      | Increase                          |
| Ecological community metrics |                                   |
| Total abundance ( $N$ )      | Increase or decrease <sup>a</sup> |
| Taxa richness ( $T$ )        | Decrease <sup>b</sup>             |
| % dominance                  | Increase                          |
| Simpson's diversity ( $D$ )  | Decrease                          |
| Shannon–Wiener ( $H'$ )      | Decrease                          |
| Community function metrics   |                                   |
| % collector-gatherers        | Increase                          |
| % collector-filterers        | Increase                          |
| % total collectors           | Increase                          |
| % scrapers                   | Decrease                          |
| % shredders                  | Decrease                          |

<sup>a</sup> Moderate degradation may cause abundance to increase.

<sup>b</sup> In mountain streams, some forms of disturbance may cause  $T$  to increase.

of crayfishes as bioindicators at this time (North Carolina Department of Environment and Natural Resources 2003; Peake et al. 2004). Crayfish can accumulate high concentrations of metals and organophosphates and are indicators of the presence of these compounds (Mirenda 1986; Alikhan et al. 1990; Young and Harvey 1991). Similarly, as filter feeders, freshwater mussels are sensitive indicators of the presence of metals and other contaminants (Adams et al. 1981; Turick et al. 1988; Martel et al. 2003; Hull et al. 2006); moreover, because many species are long-lived, their shells can provide a historical record of environmental contamination (Green et al. 1989; Brown et al. 2005). Strength of mussel recruitment can also be highly sensitive to environmental conditions (Payne and Miller 2000), but reference conditions for this potential metric are currently unquantified.

## 10.7 CONCLUSION

Aquatic plants and invertebrates represent an extremely diverse group of organisms that require diverse sampling and study approaches. Many common sampling methods can provide

data simultaneously on a wide variety of organisms. However, the choice of sampling gear and methodology is ultimately dependent on the goals of the study because each method has its own advantages and disadvantages. Qualitative methods are generally faster and less expensive and allow greater coverage of habitats or landscapes. These methods are most appropriate for surveys of diversity or species distributions at large scales or for collecting organisms for laboratory studies or field experiments. Quantitative methods are usually more laborious but can produce precise, repeatable estimates necessary for describing spatial or temporal patterns in abundance or biomass. A combination of qualitative and quantitative methods can maximize the effectiveness of studies by incorporating strengths of both approaches. Knowledge of the natural history of the target organism is essential in selecting a sampling method and ensuring that sampling occurs in the appropriate habitat and at the appropriate time.

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