Chapter 9
Collecting, Processing, and Identification of Fish Eggs and Larvae and Zooplankton

WILLIAM E. KELSO, MICHAEL D. KALLER, AND D. ALLEN RUTHERFORD

9.1 INTRODUCTION

This chapter is an introduction to methods for collecting, processing, and identifying planktonic animals that typically inhabit pelagic, benthic, and macrophytic habitats. It also summarizes the diverse studies on ichthyoplankton and zooplankton in both marine and freshwater environments. We review the gears that are used to collect these organisms, their relative effectiveness in different sampling situations, and the potential effects of aquatic physicochemistry (e.g., turbidity, dissolved oxygen concentrations, and habitat complexity) and organismal behavior (e.g., vertical migration, phototaxis, and gear avoidance) on sampling design. We also discuss sample preservation and processing, as well as the terminology, techniques, and taxonomic guides used in the identification of zooplankton and fish eggs and larvae.

Early investigators studying the growth, reproduction, and mortality of fish populations documented the critical importance of early life stages to overall abundance (Hjort 1914). Fishes are relatively fecund vertebrates, but most populations exhibit high egg and larval mortality (>90%) and significant year-to-year variation in early life stage survival (Nash and Dickey-Collas 2005; Straile et al. 2007) that can ultimately influence recruitment to adult stocks (Beaugrand et al. 2003; Head et al. 2005). Ichthyoplankton mortality is usually attributed to inherited defects, egg quality, starvation, disease, predation, and environmental fluctuations (Pepin et al. 2002; North et al. 2005; Zeldis et al. 2005). Periods of high mortality are often associated with critical events in early ontogeny (e.g., hatching, first feeding, or initiation of swim bladder function; Nislow et al. 2004; Armstrong and Nislow 2006), and the timing and duration of these critical periods may be closely tied to environmental variability and zooplankton prey abundance.

Relationships among fish early life stages, stock abundance, and fisheries harvests have been the basis for numerous studies on the abundance, distribution, ecology, and dynamics of fish eggs and larvae (e.g., Werner and Fuiman 2002; Dege and Brown 2004; Miller and Shanks 2005). A comprehensive review of the larval fish literature is beyond the scope of this chapter, but examples of larval fish research illustrate the diversity of questions that have been addressed. Egg and larval collections have been used to identify spawning and nursery areas (Ådlandsvik et al. 2004) as well as spatiotemporal differences in spawning characteristics of exploited populations (Bellier et al. 2007). Larval fish studies have also yielded important information on ontogenetic changes in movement patterns (Zitek et al. 2004a, 2004b; Hare et al. 2005), foraging behavior (Puvanendran et al. 2004), and condition (Catalán et al. 2007). Larval fish growth may be closely linked to larval survival rates (McCormick and Hoey 2004), and growth studies typically involve analysis
of daily ring patterns in larval otoliths (Miller and Storck 1982; Bergenius et al. 2005; Uehara et al. 2005; see Chapter 15), as well as assessments of RNA:DNA ratios (Buckley 1984; Buckley et al. 2004; Caldarone 2005; Tardif et al. 2005). Additional larval fish studies have addressed topics such as physiology (Persaud et al. 2006), behavior (Leis et al. 2006), taxonomy and identification (Wallus et al. 1990; Snyder et al. 2004; Hyde et al. 2005; Richards 2006), systematics (Leis et al. 1997), and responses to anthropogenic stress (Humphries et al. 2002; Rudneva and Zalevskaya 2004).

Studies of larval fish trophic ecology often include collections of cohabiting zooplankton populations (e.g., Lough and Broughton 2007) to assess interactions between larval fish and zooplankton (Fossheim et al. 2006) and prey selectivity (Fulford et al. 2006; Nunn et al. 2007). In a broader context, the study of zooplankton has also been a diverse and productive field of research and has yielded a tremendous amount of information on the structure and function of biotic communities in freshwater and marine ecosystems. At the community level, zooplankton abundance can provide important information on trophic structure and dynamics (Leonard and Paerl 2005; Forrest and Arnott 2006), ecosystem function (Baranyi et al. 2002; Pershing et al. 2005), and environmental bioassessment (Hjorth et al. 2006; Derry and Arnott 2007). Additional studies have addressed the effects of various ecological interactions on population and community structure, including predation and competition (Dzialowski and O’Brien 2004; McNaught et al. 2004), dispersal (Havel and Shurin 2004), habitat characteristics (Krumme and Liang 2004; Steiner 2004), reproduction (Alekseev 2004; Varpe et al. 2007), and movement (Boeing et al. 2004), particularly the phenomenon of diurnal vertical migration (Hays 2003; Reichwaldt and Stibor 2005; Jack et al. 2006).

Because planktonic organisms differ in size, morphology, mobility, vertical and horizontal distribution, temporal availability, and susceptibility to various gears, proper collection, handling, and preservation techniques are critical to the design of an effective sampling program. Many types of active and passive collecting gears have been used to capture fish eggs and larvae (collectively referred to here as ichthyoplankton, even if they are deposited on the bottom or on submerged structures) and zooplankton in a diversity of habitats, and the sampling characteristics and potential sources of bias of each gear should be investigated carefully before final sampling protocols are implemented. The most common methods are described in this chapter, as is literature that readers can consult regarding study design, sampling design, and gear choice. Excellent diagrams and pictures of most gear types appear in Wiebe and Benfield (2003). Summaries of plankton sampling methods include Omori and Ikeda (1984) and Harris et al. (2000).

### 9.2 SAMPLING CONSIDERATIONS

Formulation of specific research objectives is the first step in study design and selection of plankton sampling methods. Budget, manpower, equipment, and time limitations will affect study design (Chapter 1) as will numerous physicochemical, ecological, biological, and statistical considerations. For ichthyoplankton studies, information on reproductive life histories, behavior, and ecology (e.g., Hoyt 1988; Richards 1990; Carlander 1997; Scott and Crossman 1998) should be consulted when determining appropriate collecting methods, gear types, sampling periodicity, and habitats sampled (e.g., Quist et al. 2004). Zooplankton also exhibit species-specific temporal and spatial differences in abundance and distribution that will affect the study design. For freshwater taxa, general summaries of behavior and ecology can be found in Smith (2001) and Thorp
and Covich (2010), whereas information on marine zooplankton can be found in Johnson and Allen (2005).

9.2.1 Towed Plankton Nets: Effects of Sampling Characteristics on Study Design

Historically, towed nets were the primary gear used to collect plankton, and many studies have reported on gear design and performance characteristics that can significantly affect the accuracy and precision of plankton sampling programs. In addition to active gear avoidance by mobile planktonic organisms, reduced capture efficiency can also occur because of clogging of nets (Smith et al. 1968; Brander et al. 1993) or pump intakes. For towed nets and filter nets used with pumps, clogging is primarily a function of gauze material, mesh size, density of organisms and debris in the water column, and duration of sampling. Clogging can be particularly problematic during oblique (deployment to depth, followed by towing at a constant rate up through the water column to the surface) or vertical tows, as progressive clogging can lead to unequal sampling at different depths and inaccurate abundance estimates if planktonic organisms are not uniformly distributed (Schnack 1974). Comparison of flowmeters mounted inside and outside the mouth of the net can be used to assess the magnitude of clogging, which can be reduced by increasing the net area to mouth area ratio to at least 3:1 (preferably 5:1), incorporating mouth reducing cones or pre-net cylinders, and reducing tow times (Trantor and Smith 1968). Clogging is not a problem for video, digital, or acoustic samplers, although discrimination of individual organisms typically declines at high densities and low particle-to-particle distances (e.g., Remsen et al. 2004).

Damage to collected organisms can occur in high-speed samplers (towing speeds ≥ 2 m/s) and is particularly important if damage prevents their identification. Decreases in length of northern anchovy and Pacific herring of 18–19% were attributed solely to the effects of netting (Hay 1981; McGurk 1985). Moreover, effects were not consistent; body depth and head width increased as standard length decreased, resulting in inaccurate assessment of larval condition (McGurk 1985). Both damage and extrusion of collected organisms through the mesh (Vannucci 1968) are serious problems and are primarily related to the size and morphology of the collected taxa (Gregory and Powles 1988), mesh size (Hopcroft et al. 2005), tow duration, and towing speed (Nichols and Thompson 1991). Although higher towing speeds can increase damage to collected organisms, Kane and Anderson (2007) reported that higher towing speeds reduced the amount of detritus and phytoplankton collected, thereby producing a “cleaner” sample. Extrusion can be reduced with smaller-mesh nets, but smaller mesh is more susceptible to clogging and reduced filtration.

Choice of mesh size depends on gear type, water velocity through the gear, densities of clogging particles, and the size, morphology, and rigidity of the organisms being sampled. Choosing the largest mesh that will collect the desired size-classes of target organisms should maximize sampling effectiveness while minimizing clogging problems and reductions in net performance. Nevertheless, sampling small organisms in systems with large amounts of debris may require several tows of short duration. Net mesh size varies considerably among studies, ranging from less than 40–75 μm for rotifers (e.g., Molinero et al. 2006), 100–250 μm for microcrustaceans (e.g., Whitman et al. 2004; Santer and Hansen 2006), 333 to over 500 μm for freshwater ichthyoplankton (Miler and Fischer 2004; Rowe and Taumoepeau 2004; Ward et al. 2004), and 180–1,600 μm for estuarine and marine larval fishes (Dege and Brown 2004; Hare et al. 2005; Fossheim et al. 2006; Marques et al. 2006). Sampling characteristics of different mesh sizes can have significant effects on density estimates. Large-mesh high-speed samplers underestimated Atlantic mackerel egg abundances because of extrusion (Southward and Bary 1980), and large-mesh netting (500
\(\mu m\) was also responsible for losses of threadfin and gizzard shad larvae during sampling and wash-down (Tomljanovich and Heuer 1986). Losses of Great Lakes larval fishes were 26% in 1,000-\(\mu m\)-mesh nets and 13% in 480-\(\mu m\)-mesh nets, whereas all larvae were retained in 250-\(\mu m\)-mesh nets (Leslie and Timmins 1989). Significantly fewer larval alewife and rainbow smelt were collected in 0.5-m-diameter nets constructed of 450, 560, and 750-\(\mu m\)-mesh than in 355-\(\mu m\)-mesh nets (O’Gorman 1984). Mesh material may also be important, especially when analyzing historic data sets (Lenarz 1972; Pitois and Fox 2006); a change from 550-\(\mu m\) silk to 505-\(\mu m\) nylon increased retention of larval northern anchovy from 60% to nearly 100%.

If study objectives include assessment of larval fish length-frequency distributions or growth, two or more types of gears can be used to improve accuracy and reduce mechanical or biotically related bias (Suthers and Frank 1989). Gallagher and Conner (1983) used a meter net and paired 0.5-m push nets to collect fish larvae in the Mississippi River and found that the relative effectiveness of the two gears varied by habitat (main stem versus backwater) and time of day. If a study is designed to assess larval mortality (e.g., entrainment; Dempsey 1988), it is important to quantify mortality caused by sampling, which is a direct function of water velocity (O’Conner and Schaffer 1977; Cada and Hergenrader 1978; see McGroddy and Wyman [1977] for a low-mortality collection device developed for entrainment sampling).

Mesh size is also an important consideration in net-based zooplankton studies (Gallienne and Robins 2001) as taxa vary considerably in size, shape, mobility, and rigidity, and net meshes vary in their propensity to clog. Abundance estimates of major taxa calculated from data collected with 500-\(\mu m\)-mesh bongo nets were 60–100 times lower than those based on nets constructed of 335-\(\mu m\) mesh (Marques et al. 2006). With the exception of copepod nauplii, a 156-\(\mu m\)-mesh net with a filtration area to net mouth ratio of 3.06 provided the best estimates of Lake Michigan zooplankton density, with an estimated filtration efficiency of 98% (Evans and Sell 1985). A 90-\(\mu m\)-mesh net was superior to 160- and 200-\(\mu m\)-mesh nets for sampling rotifers and copepod nauplii (but not adult copepods) but was highly susceptible to clogging during periods of high phytoplankton density (Henroth 1987). A 101-\(\mu m\)-nylon-mesh net clogged 35 times as quickly as did a silk 550-\(\mu m\)-mesh net (Smith et al. 1968), and clogging was the single most important variable affecting zooplankton densities estimated from Antarctic plankton samples collected with 125-, 224-, and 270-\(\mu m\)-mesh netting (Hunt and Hosie 2006). Flowmeters should be mounted inside and outside all towed nets to determine filtration efficiency, which can vary tremendously depending on the densities of organisms such as filamentous algae and gelatin-sheathed zooplankton (e.g., *Holopedium gibberum*; McQueen and Yan 1993). Although high-speed samplers with mouth-reducing nose cones may reduce clogging (Le Fèvre 1973), specimen damage and extrusion may become problematic.

### 9.2.2 Effects of Spatial and Temporal Variability on Sampling Design

Distributions of ichthyoplankton and zooplankton vary in time and space, and this variability must be incorporated into the study design. Mating systems, egg-deposition strategies, and spawning seasons vary significantly among fishes (Potts and Wootton 1984; Murua and Saborido-Rey 2003; Snelgrove et al. 2008), and spawning activity may vary temporally both within and among years because of latitude as well as seasonal and annual variability in climate, rainfall, temperature, upwellings, and even zooplankton abundance (e.g., Coombs et al. 2006; Hodgson et al. 2006). Temporal succession of larval fishes is common (Floyd et al. 1984a; Malzahn and Boersma 2007), and although initiation, cessation, and frequency of egg and larval sampling depend on
study objectives, sampling typically commences just prior to spawning of the target species and continues at hourly (e.g., diel changes in vertical distribution) to biweekly intervals until catches cease or decline to low levels.

Zooplankton also exhibit species-specific seasonal cycles in abundance that can be strongly influenced by changes in physicochemistry (Steiner 2004; Feike et al. 2007), climate (e.g., El Niño; Keister and Peterson 2003), hydrography (Shulz et al. 2007), interspecific competition (Hülsmann et al. 2005), and trophic interactions with both predators (Dzialowski and O’Brien 2004) and forage (i.e., phytoplankton density and species composition; Durbin et al. 2003).

In addition, zooplankton can change reproductive strategies; copepods can produce diapausing eggs or early instar larvae, and rotifers and cladocerans can alternate between extended periods of clonal reproduction by parthenogenesis and brief periods of sexual reproduction that produce dormant eggs or ephippia (Alekseev 2004; Siokou-Frangou et al. 2005). Consequently, research on zooplankton population genetics (Hebert and Taylor 1997) and responses to environmental changes may involve collection and hatching of dormant stages from bottom sediments (Reid et al. 2002; Michels et al. 2007).

In addition to seasonal changes in abundance and distribution, most ichthyoplankton and zooplankton taxa exhibit short-term (e.g., diel) changes in spatial distribution related to physicochemistry, light levels, and the abundances of predators and prey. Larval fishes often move between surface and deepwater areas during a diel cycle (e.g., Hensler and Jude 2007), although migration patterns can vary substantially across taxa (Gray 1998) and even within taxa through time (Voss et al. 2007). Spatial and temporal patchiness in zooplankton distributions (Evans and Sell 1983; Roman et al. 2005) are often related to diel vertical or horizontal movements (Jack et al. 2006) that can also influence gear choice and sampling design. Diel movement patterns (Ringelberg and Van Gool 2003) may require the collection of multiple samples throughout the diel cycle to describe assemblage composition and taxa-specific population dynamics adequately (Castro et al. 2007). Littoral zooplankton taxa may also exhibit diel movements (Meerhoff et al. 2007), which may require the use of traps to determine assemblage composition in benthic and structurally complex littoral habitats adequately (e.g., Örnólfsdótitir and Einarsson 2004). Combinations of gears or single gears (e.g., traps) that are able to sample multiple habitats effectively may be needed for species that inhabit and move between pelagic and littoral habitats (e.g., Burks et al. 2002).

Regardless of the type or number of gears used, it is important that sampling duration, gear characteristics (e.g., mesh size), sampling speed, sampling depth, and diel sampling periodicity be quantified for each gear and be consistent among samples. In addition, interspecific variability in spatial distributions and susceptibility to various gear types must be considered in assessments of relative species composition of plankton assemblages. Investigators must consider whether differences in the numbers of various taxa collected reflect true relative abundances or are a result of interspecific differences in swimming ability, behavior, or microhabitat preferences.

9.2.2.1 Marine Systems

Variability in ichthyoplankton abundance through time (e.g., D’Alessandro et al. 2007) and horizontal and vertical patchiness resulting from passive or active aggregation (Gray 1998; Boyra et al. 2003; Bradbury et al. 2003; Alemany et al. 2006) can affect abundance estimates substantially (Voss and Hinrichsen 2003). Marine zooplankton are no less patchily distributed (e.g., Solow and Steele 1995), and spatial distribution patterns of all plankton depend on buoyancy and
behavior (e.g., Sclafani et al. 1993; Cohen and Forward 2005), which are affected by temperature, wind and current patterns, salinity, light, and the distribution and movement of predators and food (e.g., Coyle and Pinchuk 2005). Pelagic fish eggs may be most abundant at intermediate depths where temperature and salinity render them neutrally buoyant (Nissling et al. 2003), but in estuarine habitats, vertical egg distribution may be a function of river discharge (Marley 1983). Ichthyoplankton abundance and phenology can be closely tied to surface temperature fluctuations (Greve et al. 2005) and thermocline depth (Suthers et al. 2006) and may or may not be tightly coupled to zooplankton abundance patterns (Sanvicente-Añorve et al. 2006). Given concerns about the effects of global warming on marine ecosystem function, considerable research is currently focused on understanding the forcing factors that determine the distribution and abundance of the entire plankton assemblage. Understanding these relationships is also critical to the continued development of effective plankton sampling programs.

9.2.2.2 Rivers, Streams, and Estuaries

Ichthyoplankton abundance on inundated floodplains may be high in large river systems (e.g., Sommer et al. 2004), but floodplain use is variable among taxa and rivers, and determination of larval fish distribution may require sampling of both lotic and lentic habitats (Humphries et al. 2002; King et al. 2003). In rivers, larvae can be abundant in backwater areas (King 2004), along the shoreline (Reichard et al. 2004), or in the mid-channel water column (Smith and King 2005), with peak periods of egg and larval drift often occurring at night (e.g., White and Harvey 2003; Baumgartner et al. 2004; Zitek et al. 2004a). Within and among species, larval drift appears to be related to length-specific behavioral reactions to light (Zitek et al. 2004b) and fluctuates with seasonal changes in stream discharge and physicochemistry, particularly temperature (Johnston and Cheverie 1988), which strongly influences spawning (Smith and King 2005). Zooplankton is typically not abundant in small streams and is quickly removed by filter-feeding macroinvertebrates in outlet streams below lakes and reservoirs (Walks and Cyr 2004). However, diverse and trophically important zooplankton assemblages inhabit large river systems, although the physical (e.g., retention areas) and biological factors influencing plankton community dynamics in these systems need further study (Thorup and Mantovani 2005).

Ichthyoplankton assemblage composition in estuaries is strongly influenced by fluctuations in temperature, rainfall, and river discharge (Ramos et al. 2006a). Larval fish assemblages can vary longitudinally, with high diversity near the ocean (including species of marine origin) and low diversity, high-dominance assemblages made up of shallow salt-marsh resident species farther upstream (Ramos et al. 2006b). Larvae can exhibit behavioral responses to depth, flow, and light that permit upstream transport from the lower to the upper estuary (Shultz et al. 2003). The factors that determine the distribution, settlement, and survival of larval fishes (Able et al. 2006) and the exchange of larvae between estuaries and offshore marine habitats (Miller and Shanks 2004; Hare et al. 2005) are important areas of research. For some oligohaline fishes, the estuarine turbidity maximum (ETM) created by the interaction of saline and fresh waters may be important because of high densities of zooplankton prey (Islam et al. 2006). Sampling ichthyoplankton in these dynamic systems may be problematic, but understanding the ecology of fish early life stages in estuaries may be particularly important because of the close linkages between estuarine physicochemistry (particularly the ETM and salt front), zooplankton abundance, and larval fish distribution, which may influence fish recruitment dynamics (North and Houde 2003). Estuarine zooplankton community composition reflects diel and seasonal changes in temperature,
freshwater input, tidal surges, density, salinity, light, and turbidity, as well as anthropogenic factors such as eutrophication (Albaina and Irigoien 2007; Marques et al. 2007). Vertical and lateral movement patterns appear to be closely tied to tidal fluctuations and can facilitate either dispersal to the ocean or retention within the estuarine environment (Naylor 2006).

9.2.2.3 Freshwater Lakes

Larval fishes in freshwater lentic systems exhibit behavioral changes and habitat shifts that significantly affect sampling designs (e.g., Leslie 1986; Quist et al. 2004). Basin morphology and wind exposure can influence assemblage composition (Eggleton et al. 2005), and mass water movements in large systems affect the distribution of young larvae (Höök et al. 2006). The larvae of many species move to littoral habitats after a period of limnetic residence (Werner 1969), but spatial distributions can vary both among and within species (Conrow et al. 1990). Within the littoral zone, the relationship between larval abundance and macrophyte density can change during ontogeny (Faber 1980; Gregory and Powles 1985), probably reflecting a balance between foraging efficiency and size-mediated vulnerability to predators (Byström et al. 2003). Pelagic larvae can exhibit substantial diel movement (e.g., clupeids rising to the surface at dusk in open-water and freshwater drum moving to deeper waters at night; Tuberville 1979). Such ontogenetic changes in habitat preferences, combined with increasing size and decreasing vulnerability to various sampling gears, often bias abundance estimates of late larvae and early juveniles.

Lacustrine zooplankton assemblages are typically dominated by limnetic and littoral rotifers, cladocerans, copepods, and other organisms (e.g., ostracods), each exhibiting taxon-specific behavioral characteristics and habitat preferences that must be considered in sampling designs. Zooplankton distribution and abundance can be influenced by biotic factors such as phytoplankton assemblage composition (Kå et al. 2006), competition (Dzalowski and O’Brien 2004), predation (Romare and Hansson 2003), and their interaction (Ciros-Pérez et al. 2004). Abiotic factors such as dissolved oxygen concentration (Auel and Verheye 2007), water temperature (Johnson et al. 2007), ultraviolet radiation (Boeing et al. 2004; Leech et al. 2005), turbidity (Dejen et al. 2004) and its interaction with predation (Castro et al. 2007), internal hydrophysics (Rinke et al. 2007), and lake geography and productivity (Sweetman and Smol 2006) can also determine assemblage composition, abundance, and distribution. Extensive vertical (Ohman 1990) and horizontal (Michels et al. 2007) diel migrations have been documented for various zooplankton groups in lacustrine systems, and most appear to be related to minimizing mortality from both vertebrate (Gliwicz 1986) and invertebrate (Irigoien et al. 2004) predators. Depending on objectives of the study, such changes in distribution may need to be carefully considered in the design of zooplankton sampling programs (Jack et al. 2006) and may necessitate the use of several gears (e.g., pelagic nets and littoral traps) during both day and night to characterize zooplankton assemblage composition adequately.

9.2.3 Density and Sample Volume Effects on Sampling Design

Target sample volumes depend on study objectives but will also vary with gear type, gear size (McGowan and Fraundorf 1966), towing speed (Thayer et al. 1983), and clogging (Hunt and Hosie 2006), as well as the abundance of targeted organisms and their ability to avoid the gear (Fleminger and Clutter 1965; Brander and Thompson 1989). Sampling a large volume of water increases the probability of encountering patches of ichthyoplankton and zooplankton and of capturing mobile taxa or life stages, but large nets may be difficult to use, and extended
sampling times increase clogging. For general studies of plankton abundance, 30 m³ per sample is a good target volume in freshwater systems, whereas up to 1,500 m³ may be needed in marine plankton studies (Marcy and Dahlberg 1980). If objectives include assessment of vertical and horizontal patchiness (Castro et al. 2007), pumps or opening–closing gears can be used to filter target volumes at discrete depths (Harris et al. 1986). Traps are also effective gears for sampling at depth, but sampled volumes are usually small (<1.0 m³), and many samples may be needed to capture sufficient numbers of organisms if plankton abundance is low. Vertical, horizontal, or oblique net tows are commonly used if presence–absence or temporal abundance data are needed, whereas several high-speed towed gears (section 9.3.2) can provide small-scale discrimination of plankton abundance while still sampling a substantial volume of water.

9.2.4 Gear Avoidance

Estimates of plankton abundance can be significantly biased by gear avoidance (Clark et al. 2001), which can be both passive (organisms move with the pressure wave away from the sampler mouth, which may increase as meshes become clogged) and active (detection of the sampler and movement out of the net path). Thayer et al. (1983) suggested that reduced catches of estuarine fish larvae in high-speed samplers towed above 8 m/s were due to deflection by the pressure wave in front of the sampler, although extrusion through the collecting net was also a possibility. Similar “pressure-wave” avoidance has been suggested for high-speed zooplankton samplers (Hunt and Hosie 2003; Richardson et al. 2004), although quantifying the magnitude of this type of avoidance has been difficult.

Active avoidance of nets (or net bridles; Filion et al. 1993) and pumps (Cada and Loar 1982) has been assessed with comparisons of plankton length distributions and densities from simultaneous collections with different gear types (Clark et al. 2001; Claramunt et al. 2005; Overton and Rulifson 2007) and with comparisons of sample composition in diurnal and nocturnal samples (Graham and Venno 1968; McGurk 1992; Ianson et al. 2004). Avoidance is related to fish size, position relative to the gear, light levels, physical characteristics of the sampling gear, water velocity entering the gear, and clogging. Visual signals (Clutter and Anraku 1968) and hydrostatic pressure waves may trigger avoidance responses by ichthyoplankton that can cause significant underestimates of abundance, particularly of larger larvae. Increased catch rates of larval Hawaiian anchovy in meter-net samples collected at night in Kaneohe Bay, Hawaii, indicated that avoidance was primarily visual (Murphy and Clutter 1972). Similarly, visual avoidance of a 5-m² (mouth diameter) net accounted for most of the catch variability in diurnal samples of 25–40 mm larval Atlantic herring in the North Sea (Heath and Dunn 1990). In Lake Oneida, yellow perch and walleye larvae over 10 mm in length avoided a meter net but not a high-speed sampler (Noble 1971). Thayer et al. (1983) found that avoidance of a 20-cm bongo net towed at 2 m/s resulted in significant underestimation of 10–16-mm-long spot and 19–26-mm-long Atlantic menhaden abundances in coastal North Carolina habitats. For low-velocity gears, nocturnal sampling generally results in substantially higher catch rates than does diurnal sampling (Cole and MacMillan 1984), although this could also be caused by changes in vertical position of larvae in the water column (Marcy and Dahlberg 1980). Use of high-speed samplers can decrease active avoidance by larvae, but extrusion and damage of collected larvae may increase.

Overall, results of studies investigating plankton avoidance of various sampling gears highlight several considerations regarding sampling design and sample accuracy: (1) active avoidance
is taxon and size specific; (2) for larval fishes, active avoidance increases with size, which can affect both mortality and growth estimations (Brander and Thompson 1989); (3) increasing the mouth diameter of towed nets generally decreases active avoidance (Fleminger and Clutter 1965; Clark et al. 2001); (4) diel changes in plankton abundance may or may not reflect gear avoidance and may be due to changes in vulnerability to a gear (e.g., surface tows) caused by nocturnal changes in water column position (e.g., Jensen et al. 2003; Ianson et al. 2004); and (5) nets pushed in front of a boat may be more effective than nets towed behind a boat because of boat-generated turbulence and noise (Claramunt et al. 2005).

9.2.5 Statistical Considerations

Most plankton studies involve estimates of spatiotemporal patterns in the distribution, taxonomic composition, abundance, biomass, and size distributions of eggs, larval fish, zooplankton, or a combination thereof. Numerous factors affect the accuracy and precision of these data, most importantly the pervasive patchiness that characterizes distributions of virtually all planktonic organisms (Wiebe and Holland 1968; Leslie 1986). Spatial distribution of the organisms relative to the sampling path, the effects of sensory and swimming capabilities on gear avoidance (Richardson et al. 2004), and loss or extrusion of organisms from the gear (Leslie and Timmins 1989) can all result in significant over- or underestimation of plankton density and distribution. Gear comparisons indicate that net samples may be particularly inappropriate for some marine plankton community analyses because of the loss of fragile and gelatinous taxa (Hamner et al. 1975; Remsen et al. 2004).

Spatial and temporal patchiness within and among planktonic taxa (Dowd et al. 2004) must be considered in the sampling design. A set of observations (e.g., counts of taxa or life stages) at a single time and place (the experimental unit) constitutes a sample in most studies (Chapter 2). Alternatively, an experimental unit may be the smallest unit of the dependent variable receiving a treatment (e.g., ichthyoplankton in an estuary where an oil spill occurred) or environmental effect, which could be as simple as existing at a certain place at a given time (as above) or gradient of salinity. Sampling units are observations used to estimate statistical parameters of the experimental unit (e.g., mean and standard deviation). Inference occurs at the level of the experimental unit, so prior to sampling, the study design should consider: (1) which observations or groups of observations constitute the sampling and experimental units; (2) whether the study includes enough degrees of freedom within the sampling and experimental units (i.e., does the study have a sufficient sample size to investigate all of the explanatory variables of interest?); and (3) whether the study has sufficient replication for suitable statistical power (i.e., will the study have the ability to discern differences at desired precision?). Zooplankton studies have been singled out as frequently lacking true replication (see examples in Hurlbert 1984 and Heffner et al. 1996). True replicates (in space or time) are independent and randomly collected samples that allow for estimation of between-sample variance for a particular experimental unit (e.g., a lake, pond, or bay), which is the basis for statistical tests of significant differences in plankton abundance, species composition, and size, for example, among experimental units (Waters and Erman 1990). These concepts are explored in the example presented in Box 9.1. Consultation with a statistician or experienced researcher prior to sampling may help avoid pseudoreplication.

Data obtained from replicate plankton samples often exhibit low precision; increasing precision by maximizing (as much as is feasible) sample volumes and the number of replicates is critical (Downing et al. 1987). Cyr et al. (1992) reported that most larval fish surveys were based on low
Box 9.1 Proper Statistical Design in Plankton Studies

Designation of sampling units, experimental units, replicates, and subsamples are important issues in a plankton sampling program. For example, consider a study in which researchers are interested in determining the potential for establishment of naturally reproducing populations of an endangered fish in several lakes. The researchers believe that lakes which support the greatest zooplankton density will be the most likely to support the establishment of naturally reproducing fish populations. Ten lakes of a variety of depths within the known acceptable range for the fish are selected for this study.

The researchers define each lake as an experimental unit because zooplankton density among lakes is the comparison of interest, not the zooplankton density among parts of each lake. To collect zooplankton, the researchers plan to conduct two 10-min replicate plankton tows with a 0.5-m-diameter, 80-μm-mesh net in each lake on two different days about two weeks apart, resulting in 40 total tows (10 lakes × 2 days × 2 tows on each day in each lake). Concurrently, the researchers will measure dissolved oxygen, temperature, and chlorophyll a, which may influence zooplankton and confound inferences about the lakes. Will the researchers avoid pseudoreplication and be able to address all of their questions with this design?

Designation of each lake as an experimental unit only partially completes the statistical design. The researchers must next decide whether sampling and subsampling units exist and what these units might be. In this study, zooplankton density estimates from each sampling day could be designated as sampling units for comparisons among lakes. By sampling two weeks apart, the researchers could reasonably assume the zooplankton data were temporally independent, given the life histories of zooplankton. In this design, the replicate plankton tows would be subsampling units for estimating variability within a lake between collection days. This design has 39 df for the analyses and assumes that the plankton tows are independent. Alternatively, one could assume that the replicate plankton tows at a given depth within a given lake were not independent. Consequently, the tows might be combined, and the combined tows at a given depth of a given lake would be the sampling units for comparison among lakes, resulting in 19 df for the analyses. Because the number of degrees of freedom determines the number of “questions” one may ask of the data (i.e., the number of explanatory variables in the analysis) and the statistical power of an analysis (i.e., the precision or amount of detectable difference among habitats), it is important to identify the correct degrees of freedom. The potential for pseudoreplication by lack of independence among plankton tows may be determined by previous experience, examples from the literature, or analytical procedures such as temporal or spatial variograms, Moran’s I function, Geary’s C metric, the Durban–Watson test, or assessments of partial and inverse autocorrelation (see Rahel and Jackson 2007 for other suggestions). For the latter methods, we suggest consultation with a statistician or experienced researcher. Clearly, 39 df allows the researchers to ask more in-depth questions about the lakes and about the ancillary physicochemical variables than would 19 df. Consequently, properly designing the experiment prior to sampling and eliminating potential problems associated with pseudoreplication is important.

(Box continues)
Box 9.1 Continued

What if average lengths of zooplankton in the lakes were of interest because of gape limitations or feeding preferences of the fish of concern? In such case, the experimental units would remain the lakes, but the sampling units would now be individual zooplankters that were measured for length. Plankton tows and sampling date would now become structural variables that would be included in the statistical model (Snedecor and Cochran 1989; Noble et al. 2007) and would no longer be considered in the definition of experimental or sampling units.

numbers (≤4) of high volume (about 300 m³) replicates and that half of the published studies on larval fish abundance could detect only order-of-magnitude (i.e., 10×) differences among sites or time periods because of high variability among replicates. For example, at an abundance of 10 larvae per replicate tow, 33 replicates would have been needed to detect a 50% change in density at α = 0.05. Historically, many net-based plankton studies were based on two or three replicates at each site (sometimes confounded by pseudoreplication), with little consideration of statistical power. Although increasing the number of replicates may be problematic (depending on the gear being used), taking larger numbers of lower-volume samples could reduce the variance and improve the precision of estimated parameters and the probabilities of detecting differences among sites. However, target volumes would necessarily be dependent on the density of organisms collected to ensure the probability of capturing organisms in each sample. Moreover, care should be taken to assess the underlying distribution of plankton data prior to analyses. Most procedures used to estimate plankton abundances are based on the assumption that organisms are randomly distributed following the Poisson distribution (see Postel et al. 2000). In reality, the distribution of plankton may be sparse (zero-rich) or overdispersed (e.g., the negative binomial distribution) because of patchiness. In addition, the data may be heteroscedastic (i.e., the variance-to-mean relationship changes across the data set), and data transformation or nonparametric treatment of the data may increase the probability of type I errors; generalized linear mixed models may offer a solution in such cases (McArdle and Anderson 2004).

Other common sources of error that may limit inference, reduce statistical power, and render confidence intervals so vast that any conclusion is problematic include subsampling and counting errors (section 9.5.1), incorrect sorting (section 9.5.2), failing to account for mass loss and morphological changes in biomass estimation (Postel et al. 2000), and misidentification (section 9.6). Study designs should minimize these sources of error prior to sample collection. Practical steps include choosing to enumerate completely rather than subsample, collecting additional samples to increase the number of organisms for abundance estimation and reduce the counting error rate, and either setting appropriate goals for taxonomic resolution, collaborating with taxonomic experts, or both.

9.3 COLLECTION OF ICHTHYOPLANKTON AND ZOOPLANKTON

The range of sizes, behaviors, morphologies, and habitat preferences that characterize freshwater and marine ichthyoplankton and zooplankton has resulted in a diverse array of collecting gears designed for specific sampling situations. Most pelagic organisms are collected by filtering
water through fine-mesh nets, whereas collection of demersal, attached, or migrating organisms typically involves the use of artificial substrates or traps. The literature is replete with papers describing traditional plankton gears that have been modified for specific sampling conditions, but it is important that the advantages and disadvantages of a particular gear be considered prior to sampling (Table 9.1). Gear choice may affect data accuracy and study conclusions (Masson et al. 2004), particularly in situations in which gear types have changed in the middle of long-term data collection programs (e.g., Ohman and Smith 1995). The following sections summarize specific types of plankton sampling gears, as well as characteristics of gears, habitats, and planktonic organisms that can significantly influence the design of the sampling program.

9.3.1 Active Collecting—Low-Speed Gears

Use of towed nets (Figure 9.1A) to collect planktonic organisms can be traced to 1828 (Fraser 1968). Many improvements in construction materials, gear design, and sampling methodology have since increased the accuracy and precision of abundance estimates (Sameoto et al. 2000) and have adapted gears for specific sampling situations. Factors affecting the choice of sampling gear include study objectives, expense, ease of use, relative effectiveness in collecting various taxa, characteristics of the habitat to be sampled, and potential sources of sampling bias.

9.3.1.1 Plankton Nets

Conical nets (Figure 9.1) with mouth diameters ranging from about 0.1 m to over 1 m have been used extensively to sample planktonic organisms. Large nets (>0.5 m in diameter) are usually towed (e.g., Carleton and Hamner 2007) by boat (see Strydom 2007 and Beldade et al. 2006 for personal watercraft and underwater scooter applications) or by hand (Grimaldo et al. 2004; Pichlová et al. 2004) at speeds under 2 m/s for periods ranging from 30 s to an hour, depending primarily on plankton density and net clogging (Hunt and Hosie 2006).

Simple plankton nets typically consist of a nylon-mesh cone or cloth cylinder and mesh cone combination attached at the proximal end to a steel or brass ring (Figure 9.1A). Nets are usually connected to the towing cable with a three-strand bridle, although Filion et al. (1993) reported that a cantilevered bridal design reduced tow-line avoidance by zooplankton. Paired nets mounted in a rigid frame attached to the towing cable are called bongo nets (Figure 9.1B). Towing briddles do not obstruct water flow through these nets, and they have been used extensively in collections of zooplankton and larval fishes (Ohman and Lavanigos 2002; Marques et al. 2006; Stehle et al. 2007). Note, however, that samples in each net are not replicates, and their use as such will result in pseudoreplication if the zooplankton assemblage at a particular point in time is the experimental unit (Heffner et al. 1996; section 9.2.5). The distal end of towed plankton nets is usually fitted with a collection bucket (Duncan 1978; Graser 1978; Figure 9.1A), into which organisms clinging to the net are washed after net retrieval. Miller (1973) used replaceable 333-μm-mesh cod end bags instead of collection buckets in a push-net system designed for lacustrine fish larvae. The collection bags produced relatively undamaged larvae (compared with 505-μm-mesh bags) and had the advantage that entire bags could be removed and preserved, reducing sampling time.

To determine sample volumes, flowmeters should be mounted in the mouth of the net (Figure 9.1; Gehringer and Aron 1968). For circular mouth nets, average velocity is best measured with the flowmeter positioned about one-fourth of the mouth diameter from the edge (Smith et al. 1968). Reduced filtering efficiency caused by clogging can be assessed with an additional flowmeter positioned outside the net (Webber et al. 2005). An open-area ratio (ratio of the open mesh filtering area to the mouth area) of at least 6:1 has been recommended for maximum filtra-
### Table 9.1 Advantages and disadvantages of ichthyoplankton and zooplankton collection gears.

Abbreviations are MOCNESS (Multiple Opening–Closing Net and Environmental Sensing System); ARIES (Auto-Recording Instrumented Sampler); CPR (Continuous Plankton Recorder); LHPR (Longhurst–Hardy Plankton Recorder); VPR (Video Plankton Recorder); SIPPER (Shadowed Image Particle-Profiling Evaluation Recorder); OPC (optical plankton counter); and LOPC (Laser Optical Plankton Counter).

<table>
<thead>
<tr>
<th>Gear type and examples</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low-speed gears</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical net tows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buoyant net</td>
<td>Reduced net avoidance by organisms; useful in shallow, vegetated habitats</td>
<td>Small volume filtered; integrated water column sample</td>
</tr>
<tr>
<td>Hensen net</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal net tows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meter net</td>
<td>Large volume filtered; relatively inexpensive; capacity to be towed or anchored; only small vessels required; adaptable for pulling, towing, or pushing</td>
<td>Clogging and reduced filtering efficiency may vary with water turbulence; active and passive avoidance by organisms</td>
</tr>
<tr>
<td>Benthic sled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tucker trawl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuston net</td>
<td>Reduced net avoidance by organisms; collected organisms in good condition</td>
<td>Small sample area; extended time needed for sampling; effort difficult to quantify</td>
</tr>
<tr>
<td>Henson net</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purse seine</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High-speed gears</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller high-speed</td>
<td>Reduced net avoidance by organisms; reduced nose cones can increase filtration efficiency; large volume can be sampled over extensive areas; sampler instrumentation can provide simultaneous physicochemical data</td>
<td>Extrusion and damage of small and soft-bodied organisms; larger vessels and deployment gear often necessary; clogging; active and passive avoidance by organisms</td>
</tr>
<tr>
<td>Jet net</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulf VII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarke–Bumpus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOCNESS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARIES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multinet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plankton recorders</td>
<td>Continuous or discrete sampling over large area; reduced sampler avoidance by organisms; sampler instrumentation can provide simultaneous physicochemical data</td>
<td>Clogging; sampling efficiency may be dependent on sampling speed</td>
</tr>
<tr>
<td>CPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LHPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-Tow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical recorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPR</td>
<td>Capacity to record fragile taxa; real-time, small-scale data; no clogging</td>
<td>Taxonomic recognition may be problematic; inaccuracies at high particle densities because of particle coincidence</td>
</tr>
<tr>
<td>SIPPER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOPC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9.1  Continued

<table>
<thead>
<tr>
<th>Gear type and examples</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pumps</strong></td>
<td>Large volumes filtered by centrifugal pumps; effective over coarse substrates; discrete samples in time and space</td>
<td>Clogging under turbid conditions; damage to collected organisms; active avoidance by organisms</td>
</tr>
<tr>
<td>Centrifugal pumps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm pumps</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Traps</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg trap</td>
<td>Typically inexpensive; some gears passive for extended sampling; collected organisms in excellent condition</td>
<td>Small sample area or volume; quantifying abundance by volume may be difficult for gears other than tubes and Schindler traps; potential predation in traps; behavioral selectivity (e.g., phototaxis)</td>
</tr>
<tr>
<td>Emergence trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schindler trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Funnel trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light trap</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tube sampler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Point abundance”</td>
<td>Reduced avoidance by organisms; microhabitat sampling</td>
<td>Highly specialized gear; limited sample area</td>
</tr>
<tr>
<td>Electric sweep net</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9.3.1.2 Benthic Plankton Samplers

Plankton sleds that incorporate Clarke–Bumpus samplers (Clarke and Bumpus 1950; Frolander and Pratt 1962), circular plankton nets (Dovel 1964), and rectangular nets (Carleton and Hamner 2007) mounted on metal frames sample plankton on or just above the bottom (Figure
PLANKTON

Figure 9.1  Plankton nets: (A) simple cylinder–cone plankton net with a purse line for sampling at discrete depths; (B) paired bongo nets fitted with a depressor to maintain the nets at a prescribed depth; and (C) three plankton nets rigged vertically to sample drifting eggs and larvae in lotic habitats.

9.2C). Underwater obstructions and high turbidity (disturbance of bottom sediments and net clogging) can limit the effectiveness of sled-type samplers, but plankton sleds have been shown to provide better abundance estimates of fish eggs and demersal fish larvae than do standard plankton nets (Yocum and Tesar 1980; Madenjian and Jude 1985). The rectangular sled developed by La Bolle et al. (1985) included an adjustable net that could effectively fish the entire water column in depths ranging from 0.15 to 0.70 m, whereas Phillips and Mason (1986) incorporated a self-adjusting grate to sample demersal adhesive and nonadhesive fish eggs on irregular substrates. Carleton and Hamner (1987) used a diver-operated herding trap for epibenthic plankters in a coral reef lagoon. This trap has proven to be an effective gear for numerous invertebrates (Carleton and Hamner 2007) and also would probably be effective for demersal ichthyoplankton as well.

9.3.1.3 Pelagic Trawls

Low- to moderate-speed (0.5–3 m/s) midwater trawls for sampling zooplankton and pelagic fish larvae include the Isaacs–Kidd midwater trawl (Isaacs and Kidd 1953; Figure 9.2D) and its successors (e.g., the HPN, Hamburg Plankton Net; Vacchi et al. 1999). They are of simple design and have been used extensively to sample ichthyoplankton and small juveniles in pelagic areas. The steel-framed Tucker trawl (1.8 m × 1.8 m; Tucker 1951) was modified by Houser (1983) and
Figure 9.2  Towed or pushed nets: (A) vertically adjustable push net; (B) side-mounted ichthyoplankton net; (C) benthic sled for shallow-water sampling; and (D) Isaacs–Kidd midwater trawl fitted with a depressor for improved performance at depth (adapted from Meador and Bulak 1987 and La Bolle et al. 1985, with permission).

Oozeki et al. (2004; stronger frame design) to include a diving plane for maintenance of position in the water column without ballast (Figure 9.3A). Although it has been used extensively for ichthyoplankton surveys (e.g., Haldorson et al. 1993; Brown 2002; Shoji et al. 2005), the Tucker trawl was ineffective for estimating the density or size composition of pelagic reef-fish larvae, particularly of small individuals (Choat et al. 1993).

Clarke (1969) described a rectangular (2.8 m × 4 m) trawl that could be opened and closed acoustically, and Baker et al. (1973) used a similar design for a rectangular midwater trawl (RMT 1 + 8), which incorporated 1-m² and 8-m² nets that could be opened and closed (Piatkowski and Hagen 1994; Angel et al. 2007). Roe and Shale (1979) successfully used a modified RMT 1 + 8 (three nets of each size and a new cod end tube) to collect depth-specific samples down to 4,500 m. The Multiple Opening and Closing Net with an Environmental Sensing System trawl (MOCNESS; Wiebe et al. 1976) is another version of this design, incorporating nine sequentially opening and closing nets (1.0 m × 1.4 m × 6.0 m long, 333-μm mesh) as well as sensors to monitor depth, temperature, specific conductance, flow, net angle, and net deployment. The MOCNESS sampler has been used extensively for depth-specific marine ichthyoplankton sampling (e.g., Auth and Brodeur 2006; Fossheim et al. 2006; Rowlands et al. 2006) and is comparable to a standard cylinder–cone net with a purse line for discrete depth sampling (Henroth 1987).
Figure 9.3  Gears for sampling specific depth strata: (A) modified Tucker trawl, and (B) neuston net for sampling eggs and larvae at the surface (adapted from Sameoto and Jaroszynski 1976 and Brown and Cheng 1981, with permission).

in total biomass collected but with increased effectiveness for larger organisms such as krill and amphipods (Gjøsæter et al. 2000). Sameoto et al. (1977) incorporated a depressor and rigid net frames in a 10-net (1-m² mouth area, 243-μm mesh) sampler (Bedford Institute of Oceanography Net and Environmental Sampling System, or BIONESS) that could be towed at speeds up to 3 m/s. The BIONESS net was superior to the Tucker trawl for sampling small (<10 mm) Atlantic cod larvae, whereas the trawl was more effective for larger (>10 mm) larvae and juveniles (Suthers and Frank 1989). The Auto-Recording Instrumented Sampler (ARIES) system incorporates serial opening and closing nets, a water sampler, and an oceanographic sensor unit (Dunn et al. 1993); more recent versions include the Hydro-Bios Multinet, which incorporates five to nine nets (depending on net diameters) that can be towed horizontally (with a depressor) or vertically (Macnaughton et al. 2007).

9.3.1.4 Neuston Nets

Studies of neustonic (near surface) ichthyoplankton and zooplankton (e.g., Reese et al. 2005; dos Santos et al. 2007) have resulted in the development of nets that are towed with the top edge at or above the water surface (Hempel and Weikert 1972; Lippincott and Thomas 1983; Figure 9.3B). A 4.9-m-long neuston net (pipe frame 2 m wide × 1 m high, 947-μm mesh) towed at
speeds from 1 to 3 m/s was effective in providing relatively undamaged specimens (Eldridge et al. 1978). Hettler (1979) modified the net by mounting it on a steel frame under a bridge for stationary sampling in a tidal current and incorporated a wooden collection box for retrieval of live larvae. The Manta net included fixed wings and asymmetrical towing cables to maintain the net at the surface away from the boat (Brown and Cheng 1981). The net was superior to other neuston nets for sampling in choppy waters (>10-cm waves), although a larger (3.5 m wide × 1 m deep) neuston trawl was more effective for larger juveniles (Shenker 1988). The Manta net appears to be particularly effective for positively buoyant and neustonic plankton and has been used extensively in near-surface plankton surveys (e.g., Moser et al. 2001; Morgan et al. 2005; Courtney and Severin 2007). Push nets (plankton nets mounted on the bow of a boat) are also effective at collecting near-surface larvae and were superior to oblique tows for sampling diadromous fishes in the Roanoke River (Overton and Rulifson 2007).

9.3.2 Active Collecting—High-Speed Gears

9.3.2.1 Nets

Conical plankton nets mounted inside hollow cylinders fitted with mouth-reducing nose cones (Figure 9.4) and arrays of electronic monitoring devices (Nash et al. 1998) are used as high-speed (>2.5 m/s) samplers in studies of both marine and freshwater ichthyoplankton (Gehringer and Aron 1968; Wiebe and Benfield 2003). High-speed samplers reduce net avoidance by mobile organisms (although avoidance can still occur; Bjørke et al. 1974; Clark et al. 2001) and can sample large volumes of water at specific depths (Swain and Roijackers 1985) over extended distances in short periods of time. The Gulf 1-A high-speed sampler (12-cm-diameter tube, 4-cm opening; Arnold 1952) was modified by Smith et al. (1964) to sample

![Figure 9.4](image-url)  
**Figure 9.4** High-speed samplers: (A) a cutaway of the Gulf III sampler (note the fore and aft flowmeters); (B) exploded view of a Miller high-speed sampler; and (C) schematic diagram illustrating the internal layout of the Continuous Plankton Recorder (adapted from Gehringer 1952; Miller 1961; and Walne et al. 1998; with permission).
at speeds up to 9 m/s. The Gulf III net (Gehringer 1952) incorporated a 0.5-m net in a rigid housing to sample a greater volume of water than the Gulf 1-A (Figure 9.4A). Bridger (1958) reported that a reduction in nose cone diameter from 40 cm to 20 cm substantially improved net efficiency and resulted in increased diurnal catches of larval Atlantic herring. Beverton and Tungate (1967) used the Gulf III to sample larval pleuronectids, phytoplankton, and zooplankton simultaneously, and modified Gulf III nets are still used in plankton studies (e.g., Dalpadado 2006). Un-encased, high-speed nets such as the Nackthai design (Schnack 1974) and the Gulf VII/PRO-NET (Nash et al. 1998; Lee et al. 2005) reduce clogging problems and have been used in several studies of larval and postlarval fish and invertebrates (Colombo et al. 2003; Nash and Geffen 2004; Kloppmann and Ulleweit 2007).

The Miller high-speed net (Miller 1961) is a lightweight, high-speed sampler that can be used in freshwater (Hansen and Wahl 1981) and marine (Takekawa et al. 2006) plankton studies by a single person in a small boat (Figure 9.4B; see Thayer et al. [1983] for a PVC-based design). Design and operational modifications include attaching the sampler to midwater trawls for simultaneous collection of zooplankton and larval fish (Doble and Eggers 1978), fixing the samplers to side-mounted 3-m poles, incorporating an electric shocking grid in front of the samplers, using clear rather than opaque materials to construct the sampler, and increasing speed for improved sampling performance (Noble 1970). Coles et al. (1977) used a small pump to empty the contents continuously of a high-speed Miller-type net used to study spatial heterogeneity of Eurasian perch larvae. Such a design seems particularly well suited for studying vertical and horizontal patchiness in egg and larval distributions, although extrusion of small larvae may be a concern, depending on study objectives (Gregory and Powles 1988). The jet net reduces damage to collected organisms by slowing the velocity of water as it moves through the sampler into the collecting net (Clarke 1964).

High-speed samplers that can be opened and closed are used to sample at discrete depths (Bé 1962; Weikert and John 1981). The Clarke–Bumpus sampler (Clarke and Bumpus 1950; Tranter and Heron 1965) is effective for assessing depth-specific zooplankton abundance (Romare et al. 2005; Piscia et al. 2006) and uses a messenger-operated closing gate to eliminate sample contamination. Kinzer (1966) modified a Gulf III net with a messenger-activated spring-loaded closing mechanism, and Bary and Frazer (1970) incorporated a similar electrically activated closing mechanism and an improved flowmeter design on the Catcher II, a modification of the Catcher sampler (Bary et al. 1958).

9.3.2.2 Plankton Recorders

An alternate sampler for obtaining spatially discrete plankton data, the Continuous Plankton Recorder (CPR; Hardy 1936) was designed to be towed by “ships of convenience” during normal cruise operations (Figure 9.4C). This sampler incorporated a rigid cone-nosed body that continuously filtered trapped organisms onto a gauze strip, which was overlaid by a second strip, both of which were wound up and preserved in a collection box. The CPR (and its numerous modifications, see Reid et al. 2003) has been used extensively for decades to sample marine zooplankton (Brander et al. 2003; Kirby et al. 2007), particularly in the North Atlantic (Beaugrand et al. 2003; Frederiksen et al. 2006; Lewis et al. 2006; Stevens et al. 2006) and was reported to be more effective than were meter nets (Colton et al. 1961) and MOCNESS and pump samplers (Brander and Thompson 1989) for assessing the distribution of larval Atlantic herring. Colton et al. (1961) noted reduced CPR sampling efficiency at low concentrations of larvae (<0.1/m³), and
Hunt and Hosie (2006) indicated potential problems with ineffective flowmeter design, reduced filtering efficiency related to high concentrations of phyto- and zooplankton, and significant influences of ship speed on sampling volumes. However, even under the highest plankton densities recorded from the North Atlantic, filtering efficiency was reduced by only 20% (John et al. 2002), and towing speed effects on filtered volumes (from about 3.0–3.8 m$^3$ per sample) were minimal (Jonas et al. 2004). Differences in CPR sampling efficiency among copepod species have been attributed to gear avoidance (Richardson et al. 2004), but development of correction factors has allowed conversion of CPR catch data to abundance estimates for integration with other data sets in the investigation of long-term trends in North Sea plankton abundance (Batten et al. 2003; Pitois and Fox 2006).

Longhurst et al. (1966) developed the Longhurst–Hardy Plankton Recorder (LHPR) by attaching a CPR collection box to a standard plankton net fitted with flow, temperature, and depth sensors for short-duration, vertical tows. Subsequent versions (e.g., Williams et al. 1983) incorporating solutions to several sampling problems (clogging, extended residence time in the sampler prior to collection, and loss through the collecting gauze; Haury et al. 1976) have been used in studies of mesozooplankton assemblage structure and vertical distribution (Irigoien et al. 2004; Ward et al. 2006), zooplankton-mediated carbon and nitrogen fluxes (Yebra et al. 2005), larval fish–zooplankton forage relationships (Sabatés 2004; Santos et al. 2006), and fish egg and larval distribution (Coombs et al. 2001, 2004).

The U-Tow overcomes several limitations of the Hardy CPR, including its fixed sampling depth, limited space for environmental monitoring instrumentation, mesh-advancing mechanism, and mesh material (Hays et al. 1998). It can be adjusted to sample multiple depths, has filtering capacity for 50 discrete samples, has an electromagnetic flowmeter, and is designed to accept conductivity–temperature–depth (CTD) sensors as well as fluorometers, optical plankton counters (OPCs), or other environmental sensors within the sampler housing. The U-Tow has proven to be an effective high-speed (>4 m/s), variable-depth sampler with increased flexibility in housing design, mesh configurations, and software-driven fishing characteristics (Hays et al. 2001; Mair et al. 2005). However, clogging may be more likely (because of its discrete mesh advance mechanism) than with the continuous mesh advance of the CPR (John et al. 2002). Samples collected with both gears simultaneously (as well as samples collected with a U-Tow and a standard plankton net; Cook and Hays 2001) indicate substantial differences in estimated plankton abundance, requiring careful consideration of mesh size and calibration prior to sampling (Batten et al. 2003).

9.3.3 Other Active Gear Types

9.3.3.1 Shallow-Water Nets

Because shallow and structurally complex areas are not easily sampled with towed nets, several other active gears (Chapter 7) have been modified to capture plankton in these habitats. Fine-mesh (≤505 μm) dip nets can be used to collect qualitative samples of zooplankton (Havel et al. 2000) and larval fishes (King and Crook 2002) from structurally complex areas, although larval fish mobility may limit effectiveness. Fine-mesh seines can be used in areas with smooth bottoms and no vegetation (Dewey et al. 1989), and small-mesh purse seines may be particularly effective in open-water areas (Kingsford and Choat 1985; Post et al. 1995; Tischler et al. 2000). These gears are easy to use, but removal of larvae could be time-consuming and result in considerable damage to specimens. In addition, standardizing seine haul and dip-net effort is difficult (depth,
speed, habitat differences, and amount of water filtered), and analyses of data obtained with these gears should therefore probably be limited to presence or absence (e.g., logistic regression; Childs et al. 1998) or abundance categorization (e.g., 0–5, 6–20, 21–100, and so on) in the absence of careful assessment and standardization of techniques.

Other shallow-water gears incorporate nets in fixed or adjustable boat-mounted frames (Figure 9.2A). Side-mounted 0.5-m- and 1.0-m-diameter nets can be used to obtain samples of ichthyoplankton from surface waters (e.g., Tarplee et al. 1979; Hodson et al. 1981; Hermes et al. 1984), and Bryan et al. (1989) mounted paired 0.5-m nets on vertically adjustable side frames braced with support wires that permitted discrete sampling at depths up to 4 m at speeds up to 1.3 m/s. Conical nets in fixed or adjustable bow-mounted frames can also be used to collect plankton in shallow areas (Holland and Libey 1981; Meador and Bulak 1987; Tischler et al. 2000; Fontenot et al. 2001). Hedrick et al. (2005) incorporated a Plexiglas collection box in an adjustable bow-mounted meter net for collection of live larval and juvenile Atlantic menhaden. Burch (1983) developed a wheel-mounted sampler for use by a wader in shallow areas, and a diver-operated device consisting of a 0.5-m net attached to two underwater towing vehicles was used to sample larvae in shallow coastal areas (Ennis 1972).

Bagenal (1974) developed a gear that vertically sampled shallow-water areas by incorporating a buoyant ring attached to a plankton net. These nets, called buoyant or pop nets, are deployed with anchors or weighted frames that take them to the bottom. After a period of time (minutes to hours), a release mechanism allows the net to rise to the surface. Although the volume of water sampled is small, net avoidance appears to be minimal (Bagenal 1974). Pop nets can provide quantitative estimates of juvenile fish abundance in vegetated littoral habitats (Dewey et al. 1989; Dewey 1992) and have been used in studies of lacustrine and marine ichthyoplankton (Cryer et al. 1986; Urho 1996; Cooperman and Markle 2003).

Drop nets incorporate a square or rectangular frame with a surrounding net suspended along the top. The frame is set in place and later released to fall quickly to the bottom (Dewey 1992). Alternatively, lightweight frames with mesh on all four sides can be thrown to the sample location, with collected fish subsequently removed by dip nets (Kushlan 1981). Frameless nets can also be thrown and purged after sinking to the bottom (Hoagman 1977). La Bolle et al. (1985) used a rectangular drop-sampler made of clear Plexiglas to reduce visual avoidance. Drop-samplers deployed from boat-mounted booms have been widely used to study microhabitat use by marsh fish (Baltz et al. 1993; Rozas et al. 2007; Zeug et al. 2007) and would probably be particularly effective for larvae and early juveniles because of their limited abilities to detect and avoid the sampler.

### 9.3.3.2 Pumps

Centrifugal pumps have been used to collect demersal eggs and larvae and to study the spatial distribution of both zooplankton and ichthyoplankton (Aron 1958; see review in Powlik et al. 1991). Most systems involve pumping a target volume of water through an intake hose into a net (Figure 9.5) or a filtering drum (to reduce damage to collected larvae). Integration of digital flow sensors can provide precise sampling of target volumes at discrete depths in the water column (Nayar et al. 2002). Such a system has several advantages: depth of sampling and volume of water through the system (duration of pumping) can be easily controlled, discrete quantitative samples can be obtained by intermittent collection of organisms from the filtering surface, the system can be operated from a stationary or moving platform, and clogging may be minimal compared with towed nets (Møhlenberg 1987). Conversely, pumped volumes can be small, pump intakes and
filtering screens can be subject to clogging, the effective pumping area of most systems is limited to several centimeters from the pump intake (avoidance by mobile larvae such as threadfin and gizzard shad can be significant; Petering and Van Den Avyle 1988), and most larvae are killed or damaged during sampling (Gale and Mohr 1978).

Pump systems have been modified extensively to collect plankton successfully in a variety of freshwater and marine habitats. Aron (1958) reported pump collections of pelagic fish eggs in Puget Sound were similar to collections taken concurrently with a 0.5-m net, although abundance estimates of several copepod taxa differed between the two gears. A high-volume pump was particularly useful for sampling larvae and associated food organisms from discrete depths in the open ocean (Harris et al. 1986). A pump system successfully collected viable walleye eggs from Lake Erie, but pump performance was reduced over mud, silt, and sand substrates because of clogging (Manz 1964). The abundance of fish larvae (particularly those >5 mm) taken from a coastal power plant intake was significantly higher in pump samples than in concurrent samples taken with 0.5-m and 1.0-m plankton nets (Leithiser et al. 1979). Adequate pump collections of lake trout eggs and early life stages required a system that incorporated a diver-directed intake (Stauffer 1981), a design that was also used for collecting smallmouth bass larvae (Novak and Sheets 1969). Diver-operated underwater diaphragm pumps were used to sample alewife and lake trout eggs as well as age-0 sculpins (Dorr et al. 1981; Flath and Dorr 1984), and a portable, diver-operated suction device that incorporated compressed air from a scuba cylinder was effective in collecting centrarchid eggs and larvae in Arkansas reservoirs (Vogele et al. 1971). A lightweight, easily adjustable centrifugal pump system housed within a perforated cylinder collected a wide

Figure 9.5  A towable pump sampler with adjustable intake for sampling at discrete depths (adapted from Gale and Mohr 1978, with permission).
diversity of mostly undamaged demersal marine plankton (Dahms and Qian 2004). Single and multiple pump systems have also been used to study lacustrine food web structure (Moustakagouni et al. 2006) as well as the vertical microdistribution of zooplankton above a coral reef (Holzman et al. 2005). Checkley et al. (1997) studied the abundance and distribution of fish eggs with a Continuous Underway Fish Egg Sampler (CUFES), which consisted of a pump, concentrator, and laboratory OPC. Egg density estimates based on CUFES samples were linearly related to densities calculated from net samples, but the CUFES was also able to sample under adverse conditions, had a constant filtration rate, and produced continuous data useful for assessing spatial and temporal trends in egg abundances (Curtis 2004; Zwolinski et al. 2006). However, the CUFES undersampled early stage eggs of several fishes and yielded substantially higher replicate variances than did bongo net samples, suggesting that multiple sampling gears may be most effective for sampling pelagic eggs (Pepin et al. 2005).

9.3.3.3 Electrofishing Gear

Electrofishing (Chapter 8) has not been widely employed to sample fish larvae. However, battery- or generator-powered electrofishing gear is particularly well-suited for sampling fish in shallow, structurally complex areas that may not be amenable to net sampling (King and Crook 2002). Electrofishing was used successfully to sample sea lamprey ammocoetes in Great Lakes tributaries (Braem and Ebel 1961). The electrofishing unit was battery powered, with electrodes made of 20-cm-square wire-mesh dip nets mounted on 1.2-m handles; intermittent application of current was most effective in extracting larvae from their burrows. McLain and Dahl (1968) successfully collected larval sea lampreys in deeper waters by means of an electrified (pulsed DC) plankton sled. Copp and Peñáz (1988) used electrofishing gear and a “point abundance sampling” approach to collect larvae of 12 fishes ranging in length from 5 to 22 mm in floodplain habitats of the upper Rhône River. The electrofishing unit was modified to include a small (10-cm diameter) anode to create a steep voltage gradient. At 200 V and 400 Hz, the battery-charged unit created a voltage gradient ranging from 3.6 V/cm at 10 cm to 0.13 V/cm at 30 cm from the anode, which appeared to be the maximum distance at which larvae would exhibit galvanotaxis. King and Crook (2002) mounted a sweep net on the anode pole of a backpack electrofishing unit to sample larval fishes and shrimps in the Broken River, Australia; the unit captured a greater size range of organisms than did a sweep net and more individuals than point abundance sampling. Modified electrofishing gear probably deserves increased use for collection of larval and juvenile fishes, although the effects of differences in fish size, water chemistry, electrode design, voltage gradient, current level, and pulse width and shape on sampling efficiency need to be evaluated (Chapter 8).

9.3.3.4 Imaging Technology

The use of towed video (Chapter 17), digital imaging, optical, and acoustic gears to record plankton abundance and taxonomic composition (in some cases) has increased significantly in the past two decades (e.g., the Ichthyoplankton Recorder; Lenz et al. 1995). Comparisons of samples collected with the Video Plankton Recorder (VPR and VPRII; Figure 9.6; Davis et al. 1992; Davis et al. 2005) and MOCNESS sampler (Broughton and Lough 2006) indicate that integration of VPR technology with traditional net sampling has several advantages. Researchers may still need net samples for detailed studies of ichthyoplankton or zooplankton life stages, but the VPR can provide abundance estimates of fragile taxa (e.g., egg-bearing copepods and gelatinous and colonial taxa; Dennett et al. 2002), as well as higher-speed, longer-tow, real-time data on micro-
and meso-scale plankton distributions with no reduction in abundance estimation because of high phytoplankton concentrations (net clogging). The use of VPR technology also allows differentiation of detrital aggregates and zooplankton of similar sizes (Ashjian et al. 2001). Combined with various taxonomic recognition technologies (Davis et al. 2004; Hu and Davis 2005; Culverhouse et al. 2006), the VPR has been used to study plankton distributions in relation to oceanographic features (Ashjian et al. 2005), size distributions of plankton prey for development of larval fish foraging models (Lough and Broughton 2007), and plankton behavior (Gallager et al. 2004). The VPR can be integrated with acoustic systems and environmental sensors (e.g., the Bio-Optical Multifrequency Acoustical and Physical Environmental Recorder, or BIOMAPER; Wiebe et al. 2002) to examine fine-scale influences of marine physicochemistry on zooplankton abundance and distribution.

The Shadowed Image Particle Profiling Evaluation Recorder (SIPPER), a digital imaging system producing two orthogonal views of particles as they pass through a sampling tube, is capable of producing images suitable for studies of the number, size, and identity of plankton (Samson et al. 2001). Comparisons with the SIPPER revealed significant underestimation (300–1,200%) of fragile and gelatinous zooplankton by traditional net tows (Remsen et al. 2004). Improved image resolution with plankton recognition algorithms and selection techniques (e.g., Luo et al. 2004) will probably improve the accuracy and utility of SIPPER data. An underwater holographic camera (eHoloCam) provides continuous 3-D, in situ plankton images during underwater tows (Sun et al. 2007); comparisons with a simultaneously deployed OPC indicated significantly higher particle densities recorded by the OPC, probably related to high densities of nonzooplankton particles (e.g., floc and aggregates) passing through the samplers.
Although optical analysis technology (Herman 1988, 1992) has been applied to in situ studies of plankton distribution and abundance (e.g., Roman et al. 2005; Yurista et al. 2006), the OPC has limitations (Vanderploeg and Roman 2006) related to zooplankton size, discrimination of organisms and inorganic particles (Liebig et al. 2006), and particle coincidence (two particles in the light beam at the same time; Remsen et al. 2004). However, significant relationships exist between net sample and OPC-derived estimates of plankton abundance and biomass (Grant et al. 2000; Woood-Walker et al. 2000; Nogueira et al. 2004), at least over restricted size ranges and abundances of plankton (e.g., Heath et al. 1999; see also Halliday et al. 2001 for possible net-extrusion effects on calibration). This technology has been integrated with taxonomic identification of net samples (to calibrate the OPC) to address a number of methodological (e.g., ellipse model effects on zooplankton biomass estimation; Patoine et al. 2006) and ecological questions, including the effects of physical forcing factors on zooplankton abundance (Pollard et al. 2002; Gallienne et al. 2004; Suthers et al. 2006) and long-term changes in zooplankton abundance from preserved samples (Mullin et al. 2003). Recent advances in OPC technology have produced the Laser OPC (LOPC), which can provide shape resolution of smaller and larger planktonic particles at much higher densities than was possible with the OPC (Herman et al. 2004). Comparison of LOPC data with simultaneous plankton net collections indicates comparable estimates of copepod species and size-class abundances when densities of other planktonic particles (e.g., diatom aggregates) are low (Herman and Harvey 2006). Improved digital technology has also yielded the Zooplankton Visualization and Imaging System (ZOOVIS), which incorporates a high-resolution digital still camera that is capable of capturing large or small particle images, depending on sample volumes. Field tests indicate that the system can provide fine-scale spatial data on particle distribution, orientation, and identity (depending on the organism) at finer resolution than can the OPC, albeit with sequential versus continuous images (Benfield et al. 2004).

9.3.3.5 Acoustic Technology

Acoustic technology has been used to study plankton community structure and distribution (Medwin and Clay 1998; Crisp and Harris 2000), and development of improved technology and advances in computerized resolution will probably enable taxon-specific 4-D resolution of abundance and distribution (Wiebe and Benfield 2003). Doppler current profilers (Chapter 4) have been employed in acoustic surveys (Lorke et al. 2004; Jiang et al. 2007; Postel et al. 2007), as have traditional echo sounders (single, dual, multi, and split beam) that are typically employed to generate estimates of volume backscattering from zooplankton aggregations (Chapter 13). These data are processed to determine backscattering patterns (organism size distributions) and individual target strengths, which can produce target densities or biovolumes by size-class (Greene et al. 1989; Pieper et al. 2001) and can be analyzed to differentiate signals produced simultaneously by fish and plankton (Korneliussen and Ona 2002). Size discrimination of plankton is frequency dependent; higher frequencies permit discrimination of smaller organisms but reduce the effective field of detection. Additional in situ data on sound movements in water relative to planktonic organisms are needed for improved quantification of plankton biomass and abundance (Wiebe and Benfield 2003). Evidence exists that Doppler current profilers do not provide accurate estimates of zooplankton dry weights in mixed-species assemblages and that backscattering accuracy is strongly taxon specific (Fielding et al. 2004). In addition, discrimination of signals from small fish larvae and invertebrates may be problematic, resulting in overestimation of larval abundance (Rudstam et al. 2002). However, combining acoustic surveys with concurrent net, pump, or opti-
cal sampling methods can provide rapid, spatially complex information on zooplankton (Trevorrow et al. 2005; Yahel et al. 2005; Lavery et al. 2007) and ichthyoplankton (Bonanno et al. 2006; Winter and Swartzman 2006) distributions. Multiple-gear comparisons have demonstrated the utility of acoustic technology for studying plankton ecology (De Robertis 2001; Sutor et al. 2005), particularly the seasonal and vertical discontinuities in plankton distributions in both marine (Pieper et al. 2001; Lawson et al. 2004) and freshwater (Lorke et al. 2004) systems, as well as simultaneous assessments of turbulence and plankton distribution (Ross et al. 2007).

9.3.3.6 Other Active Sampling Methods

Unique situations require other methods to sample various planktonic organisms effectively. A simple polyethylene bag sampler was effective for rapidly collecting replicate 1-L samples of vegetation-dwelling littoral copepods (Frisch and Wohltmann 2005). Collection of epiphytic eggs or larvae may require clipping and examination of submerged macrophytes (e.g., Pacific herring; Hoshikawa et al. 2004). Similarly, rocks or bottom debris can be collected to sample demersal eggs (Yamahira 1997). Demersal organisms (e.g., eggs and larvae of sculpins and darters and zooplankton eggs and ephippia) in or on the substrate can also be collected with dredges, epibenthic sleds (Blomqvist and Lundgren 1996; Viitasalo 2007), or corers (Madhupratap et al. 1991; Chen and Marcus 1997), although damage to fish larvae from these gears can be substantial. Eggs and early larvae of benthic-nesting fishes can sometimes be retrieved with small suction devices such as pipettes or slurp guns (e.g., Davies and Ramsey 1989). Snorkeling, scuba, or underwater video (Chapter 17) can provide data on fish spawning locations, egg deposition and abundance (McGurk and Brown 1996), larval behavior (Kääriä et al. 1997), and epibenthic zooplankton abundance (Heidelberg et al. 2004).

Collection of whole-water samples, followed by fixation and enumeration of settled organisms, has proven to be an effective method for many zooplankton species (May and O’Hare 2005). This approach can be used in vegetated littoral habitats (Pennak 1962) and is particularly effective for rotifers and other small plankton that would require fine-mesh plankton nets, which are susceptible to clogging and reduced filtration efficiency. Whole water samples can be collected with tube samplers (integrated water column or discrete water depth samples; Knoechel and Campbell 1992; Griffin et al. 2001; Gaedke et al. 2004; Iglesias et al. 2007) or discrete-depth gears such as the Ruttner (Dubovskaya et al. 2005) or Van Dorn (Figure 4.5, this volume; Morales-Baquero et al. 2006) samplers. Tube samplers are easy to use and produce estimates of integrated water column zooplankton abundance comparable with those from plankton traps and net tows (Figure 9.7; DeVries and Stein 1991).

9.3.4 Passive Gears

9.3.4.1 Drift Samplers

Many types of samplers passively collect ichthyoplankton (e.g., Tonkin et al. 2007) and zooplankton (Campbell 2002) as they drift with prevailing currents in freshwater and marine environments (Pitlo 1989). In lotic freshwater systems, anchored mesh traps (Skoglund and Barlaup 2006), inclined plane traps (Seiler et al. 2004), and drift nets (Johnson et al. 2006) consisting of plankton nets attached to circular, rectangular, or triangular frames have commonly been used to capture drifting fish eggs and larvae passively (Figure 9.8A). Schmutz et al. (1997) incorporated six nets within a rotating basket to collect discrete samples during sequential periods. Importantly, the horizontal and vertical location of drift nets in the water column must be carefully considered.
in relation to the drift characteristics of the species under study (Carter et al. 1986; Winnell and Jude 1991). Various configurations of nets placed on the bottom (Slack et al. 2004; Lecchini et al. 2005), at the surface (Zitek et al. 2004b), or in multiple positions (Tonkin et al. 2007) have all been effective for a wide variety of lotic ichthypoplankton. Mesh sizes depend on the size of the target organisms and clogging tendencies but typically range from 116 μm (Lindsay and Radle 1978) to over 1 mm (Graham and Venno 1968). As with towed nets, flowmeters should be mounted inside and outside the net mouth to estimate filtered volumes and filtration efficiency, which can decline substantially as meshes become clogged with drifting debris. Stream characteristics related to clogging, net position, and changes in flow during the sampling period should be considered in the sampling design (Faulkner and Copp 2001).
Figure 9.8  Passive collecting gears: (A) a plankton net attached to a vane for sampling in tidal currents; (B) a channel net for collecting larvae in flowing currents; and (C) and (D) emergence traps for demersal larvae (adapted from Graham and Venno 1968; Lewis et al. 1970; Porter 1973; and Collins 1975; with permission).

Drift nets can be attached to fixed poles at multiple depths, permitting deployment through the ice during winter (Winnell and Jude 1991). A net resembling a bag seine (3-mm mesh in the wings, 500-μm-mesh bag) held stationary by a 1-m × 3-m frame that could be moved up and down in the water column on fixed poles was used to capture Atlantic menhaden in estuarine channels (Figure 9.8B; Lewis et al. 1970). A drift net attached to a swivel-mounted vane was used to sample larval Atlantic herring in tidal areas of the Gulf of Maine (Graham and Venno 1968). The weight of the cod end of the net collapsed the net at slack tides, preventing escape of larvae, and the vane ensured that the net was aligned with the current when tides were flowing (Figure 9.8A). Similarly, mussel larvae were collected in tidal currents with plankton nets fitted with anterior reducing cones (Franzin and Harbicht 1992) that were mounted with
swivels on a vertical tether line (Dobretov and Miron 2001). This configuration allowed the nets to continue sampling regardless of tidal direction and prevented clogging and backwashing. Dahms and Qian (2004) used a drift net mounted within an aluminum frame on a vertically adjustable rod fitted with a vane to maintain position into the current to collect demersal marine plankton.

9.3.4.2 Traps

A variety of egg traps have been widely used to capture demersal eggs spawned in the water column or on or within the substrate. Examples include wooden frames fitted with fiberglass screen bottoms and 6.4-mm screen tops to reduce predation (Gammon 1965), artificial containers for cavity spawners (Moy and Stickney 1987), slate tiles (Downhower and Brown 1977), acrylic cylinders with mesh bottoms (Yamahira 1996), stacked plastic plates for crevice spawners (Fridirici and Beck 1986), angle iron frames filled with latex-coated animal hair (Johnson et al. 2006), and plant material attached to PVC mats (Polte and Asmus 2006). Demersal lake trout eggs have been collected with substrate-filled buckets (Stauffer 1981), nets (Horns et al. 1989), and egg traps (Marsden et al. 1991) strung together on collection lines (Ellrott and Marsden 2004). Egg traps yielded greater numbers and percentages of undamaged eggs than did nets (Marsden et al. 1991).

Larvae of demersal spawners that deposit eggs on or in gravel substrates can be captured as they emerge. Phillips and Koski (1969) used a covering net with an attached collecting bag to sample emerging coho salmon larvae, a design that was also used by Riley and Moore (2000) to study emergence of Atlantic salmon. Porter (1973) designed an oval-shaped mesh and canvas trap with a downstream collecting box to reduce water velocity (Figure 9.8C); survival of captured rainbow trout fry was 100%. The Porter trap was used to study emergence of Atlantic salmon (Gustafson-Marjanen and Dowse 1983), and a larger version successfully captured emerging Chinook salmon (Field-Dodgson 1983). Pyramidal emergence traps (Figure 9.8D) can capture larvae of both salmonids that spawn in redds and demersal-egg broadcast spawners such as lake whitefish (Collins 1975; Stauffer 1981). A similar mesh-funnel emergence trap was effective for collecting lake trout fry and was less expensive and lighter than were traditional salmonid fry traps (Chotkowski et al. 2002).

Trap design for emergence studies will depend on the species being studied (e.g., trap size), as well as the physicochemical characteristics of the system (e.g., water velocity and substrate composition). Temporal emergence patterns of the target fishes may also be important in the design of trapping studies. Most salmonid larvae emerge at night (but see Bardonnet and Gaudin [1990] for grayling), with the bulk of emergence occurring over restricted (ca. 10 d) periods (Gustafson-Marjanen and Dowse 1983; Brännäs 1987). More importantly, within-gravel movements (de Leaniz et al. 1993) can significantly bias results unless traps are large relative to the magnitude of lateral movements or trap aprons are buried deep enough in the substrate to minimize within-gravel dispersal.

Minnow-type traps (two mesh cones mounted inside a mesh cylinder) were used by Baugh and Pedretti (1986) to collect 8–60-mm fish in a shallow desert spring, and a pit trap dug into a marsh was effective for collecting juvenile mummichog from 5 to 40 mm in length as they moved with the receding tide (Kneib and Stiven 1978). Alternatively, clear Plexiglas activity traps with removable wings that direct fish to an interior slot (Figure 9.9A; Breder 1960) are inexpensive, easy to build, and can be modified for various sampling conditions (Casselman and Harvey 1973;
Trippel and Crossman 1984). The size of the entrance slot can be adjusted to capture small larvae or larger juveniles, and traps can be fished at various depths and positions in the water column depending on the behavior of the target fishes. Small versions of fyke (Beard and Priegel 1975) and trap (Beamish 1973) nets have also been used to sample larval and juvenile fishes in lentic systems. Plexiglas or net traps are particularly appropriate for vegetated habitats if target organisms are mobile and tend to move laterally along a barrier. However, comparison of catch per unit effort data among species may not accurately reflect relative abundance because of interspecific differences in trap susceptibility related to mobility, behavior, and microhabitat preferences.

Traps have been used extensively to sample freshwater and marine zooplankton and are particularly useful when depth-specific data are needed or when excessive turbidity or complex habitat structure make net sampling problematic. In addition, the species and size composition of zooplankton collected with traps may be more representative than that collected with towed nets, particularly when active avoidance of nets or extrusion of small zooplankton through the mesh occur (Kankaala 1984). The Schindler–Patalas (Schindler 1969; Borchering et al. 2006; López et al. 2007) and Juday traps (Pace and Orcutt 1981) are widely used (Figure 9.9B). Both gears
involve a vertically deployed box with doors that can be shut when the desired sampling depth has been reached, with the enclosed sample filtered through a collection bucket upon retrieval. A recent extension of this design is the Sea Core Sampler, a 1-m-high, 23-cm-wide, rectangular sampler constructed of clear PVC and used to study plankton behavior and marine snow aggregates in situ after retrieval of the isolated water volume (Kiørboe 2007).

Funnel-type migration traps have been used to sample plankton moving vertically from marine benthic habitats (Alldredge and King 1977; Hammer 1981) and horizontally into rocky-shore intertidal habitats (Setran 1992; Castilla et al. 2001; Yan et al. 2004; see Todd et al. [2006] for a cylindrical multi-baffle trap incorporating a urea and seawater killing chamber for intertidal barnacle larvae). These types of traps have also been used in freshwater littoral habitats to study zooplankton assemblage composition (Whiteside and Williams 1975; Hann and Turner 2000; Einarsson and Örnólfsdóttir 2004) and vertical and horizontal movements (Cerbin et al. 2003; Wojtal et al. 2003). Traps can be set for extended periods (e.g., 72 h; Castilla et al. 2001) but are typically deployed for several hours or overnight to capture zooplankton during crepuscular or nocturnal migrations. In all of these traps, plankton move through a funnel into a collection jar, where they remain trapped until the sampler is retrieved. Benthic re-entry traps, consisting of trays filled with clean substrate that are set in the evening and retrieved in the morning, are also effective for collecting descending zooplankton (Cahoon and Tronzo 1992).

9.3.4.3 Light Traps

Light traps are used to sample larval fish in both marine and freshwater habitats. They select for positively phototactic taxa, and catches can vary with lunar phase and tidal amplitude (Hickford and Schiel 1999; D’Alessandro et al. 2007), current velocity (Lindquist and Shaw 2005), trap size (see Hernandez and Lindquist [1999] and Meekan et al. [2001] for contrasting results), spectral characteristics of the light source (Marchetti et al. 2004), and depth of deployment (Fisher and Bellwood 2002). However, they can be placed in structurally complex habitats that preclude effective net sampling (e.g., macrophyte beds; Humphries et al. 2002), they allow sampling over extended time periods, which can increase capture probabilities, and they are often effective for more mobile, late postlarvae and early juveniles, which may not be susceptible to towed gear (Hernandez and Lindquist 1999; Miller and Shanks 2005; D’Alessandro et al. 2007). Numerous designs exist, but most include a clear plastic container with inlet funnels or slots that facilitate entrance into, and inhibit exit from, the sampler, with a mesh-walled collection bucket to filter the trap contents as the sampler is retrieved. Light sources include battery or land-line powered lights (Stobutski and Bellwood 1997; Miller and Shanks 2004) as well as Cyalume light sticks.

Dennis et al. (1991) used a light mounted above a lift net to assess abundances of ichthyoplankton in reef, sea grass, and mangrove habitats. Paulson and Espinosa (1975) used cylindrical wire-mesh (6.4-mm mesh) light traps to collect juvenile (≤40 mm) threadfin shad from limnetic scattering layers in Lake Mead, Nevada, and Kindschi et al. (1979) used a similar design to assess spatial and temporal trends in ichthyoplankton abundance in Rough River Lake, Kentucky. Faber (1981) designed a box-shaped Plexiglas light trap (Figure 9.9C) that was used to determine seasonal abundance patterns of larval fishes in vegetated habitats of two Canadian lakes (Faber 1982; Gregory and Powles 1985). In both studies, collected taxa represented about 50% of the species in the lakes, and differences in trap susceptibility existed among species and larval developmental stages. Muth and Haynes (1984) developed a smaller, floating light trap that incorporated Plexiglas leads to guide larvae to the trap entrance slots. The trap effectively sampled larvae.
and juveniles from 11 to 60 mm and captured three taxa not found in concurrent seine samples. Fisher and Bellwood (2002) incorporated horizontal baffles in cylindrical light traps to minimize vertical light scattering. The relative abundances of larvae in vertically deployed three-trap sets revealed significant differences in the vertical distribution of larvae of at least 32 species of Great Barrier Reef fishes. A vertical three-chambered trap (sequential flashing of the centrally located lights caused larvae to move through baffles from the upper entrance chamber to lower chambers; Meekan et al. 2001) was enhanced by adding underwater speakers that emitted reef sounds during sampling (Simpson et al. 2004). Catches were significantly higher in sound-enhanced traps than in silent traps.

The quatrefoil trap incorporates a central light-distributing rod surrounded by four Plexiglas cylinders milled to 3/4 of a full circle (Figure 9.9D; Floyd et al. 1984b). Advantages of this design include large trapping slots relative to the size of the trap and easy adjustment of trap size (length of the Plexiglas cylinders) and sampling depth. The quatrefoil trap collected larvae and juveniles of 25 of 28 taxa in a small Kentucky stream compared with 21 in seine hauls and 11 in drift nets; the light trap was particularly effective for cyprinid larvae (Floyd et al. 1984a). Similarly, quatrefoil traps were better than a fine-mesh seine for capturing proto-, meso- and metalarval fish in the Rio Grande River, although juveniles of some species were not present in light trap samples (Pease et al. 2006). The quatrefoil trap was modified by Secor et al. (1992) to include a chemical light source, flotation, and a collection bucket and was effective for pond-reared larval and juvenile striped bass 7–35 mm in length.

Because of differences in movement patterns, microhabitat preferences, and phototactic behavior among species, light traps are probably best suited for determination of species presence, as opposed to estimates of species relative abundance, without simultaneous assessment of species-specific light trap selectivity (i.e., concurrent sampling with a different gear). Light traps are useful for investigation of intraspecific patterns of temporal or spatial abundance (Doherty 1987), but changes in phototactic behavior with increasing size (e.g., Bulkowski and Meade 1983) must be considered in interpretation of temporal data. Light traps may be particularly effective for early larval stages (but see Doherty [1987] and Choat et al. [1993]) and typically provide larvae in excellent condition if traps are checked at frequent (e.g., 1 h) intervals (Faber 1981). Light traps were more effective than were Miller high-speed nets for 2.5–7.5-mm Iowa darter larvae (Gregory and Powles 1988), and light traps collected 200 times as many larvae and twice as many taxonomic groups as activity traps and benthic sleds in the Kanawha River, West Virginia (Niles and Hartman 2007). It is important to enumerate and identify all organisms captured in light trap samples; our experiences indicate that, depending on entrance slot width, light traps will also collect high densities of juvenile fishes and predaceous aquatic insects, both of which may significantly affect the number of larval fishes retrieved from the sampler if collections are made over extended periods such as overnight sets. An alternative would be to use light as an attractant and then use nets (dip nets, plankton nets, and fine-mesh seines) to sample attracted larvae at shorter intervals. Rooker et al. (1996) used a system with a plankton net suspended below a floating light source to collect larval fishes in inshore habitats in Puerto Rico and reported that maximum larval abundance in the net occurred within 10 min of illumination.

9.4 SAMPLE PRESERVATION

Maintaining the morphological integrity of eggs, larvae, and zooplankton is critical to ensure later utility of the samples for taxonomic and ecological studies. Just as sampling gear choice
affects physical damage to specimens, inadequate or incorrect sample preservation may lead to frustration and wasted effort. Chemical fixatives should prevent microbial degradation, minimize autolysis and cellular damage caused by osmotic changes, prevent distortion from spasmodic muscle contractions and shrinkage, and maintain melanophore pigmentation and length–mass relationships (Jones 1976; Kimmerer and McKinnon 1986; Sayers 1987; Smith 2001). Developmental stage, chemical concentration, pH, and osmotic strength can influence shrinkage and structural or pigment deterioration (Hay 1982; Tucker and Chester 1984). Generally, selection of a fixative agent balances the need to prevent microbial-induced degradation with acceptable levels of fixation-induced alterations in size, shape, and pigmentation.

9.4.1 Fixation and Preservation

After collection, zooplankton and ichthyoplankton samples will typically be fixed (stabilization of the tissue proteins so that organisms will maintain their morphology) and preserved (allowing storage without further degradation) for future study. Care must be taken, as the choice of fixative may limit specimen utility for additional analyses. For example, aldehyde-based solutions cause genetic damage (Bucklin and Allen 2004), and alcohol and aldehyde-based solutions and freezing cause morphological distortions (De Bernardi 1984; Sayers 1987; Armstrong and Steward 1997; Johnston and Cunjak 1999). Therefore, the choice of fixative should be guided by the intended use of collected specimens and should be recorded for subsequent safe handling of the specimens and assessment of specimen distortions. Regardless of the choice of fixative, however, samples should be treated immediately upon capture to ensure specimen integrity (Ahlstrom 1976; Hay 1981).

Collected larval fishes (but not eggs or zooplankton) may be subject to Institutional Animal Care and Use Committee (IACUC) guidelines for euthanasia prior to field or laboratory processing (see guidelines published by the American Veterinary Medical Association and the U.S. Department of Health and Human Services Public Health Service). Most larval fish will probably be moribund when they are removed from towed gears (Cada and Hergenrader 1978), and immediate fixation may be appropriate. If larvae are collected live (e.g., trap samples), then submersion in an ice bath until all larvae have ceased moving will probably suffice as a euthanatization technique (Wilson et al. 2009). However, IACUC protocols can differ among state and federal agencies, and it is best to consult organizational guidelines as the sampling program is being designed.

9.4.2 Fish Eggs and Larvae

Aldehyde-based solutions such as 10% formalin (4% formaldehyde) and glutaraldehyde are excellent for fixing ichthyoplankton because these solutions combine with tissue proteins and prevent them from reacting with other reagents (Pearse 1968; Lavenberg et al. 1984; Postel et al. 2000). These effects can be partially reversed by washing in water, so washing larvae after fixation prior to sorting or transfer to alcohol solutions for long-term preservation is not recommended. The transfer of formalin-fixed specimens to alcohol (usually 70% ethanol; 40–50% isopropanol may be adequate) should be done in steps of 10–20% concentration to minimize shrinkage and morphological distortion as the specimens are dehydrated. Begin the process by draining the formalin fixative and, without washing, adding the first solution in the stepped series. Repeat the process at intervals of several hours to a day per step. Although alcohol is sometimes used as both a fixative and a long-term preservative (e.g., DeLeon et al. 1991), prior fixation with an aldehyde-based solution is recommended to reduce shrinkage and deformation caused by alcohol-induced dehydration. Formaldehyde is typically preferred to glutaraldehyde as a fixative because it is less
noxious and expensive and has superior long-term stability (Steedman 1976). Aldehyde-based solutions are generally advantageous for taxonomic studies and estimation of abundance and biomass because they cause less mass loss and morphological distortion (Steedman 1976; Giguère et al. 1989; Beladjal and Mertens 1999; Postel et al. 2000). However, these fixatives should be avoided for genetic (Bucklin and Allen 2004) or stable isotope studies (Feuchtmayr and Gray 2003) in favor of freezing.

Traditionally, oocytes were fixed and preserved in 4–10% formalin or modified Gilson’s fluid (100 mL 60% methanol or ethanol, 880 mL of water, 15 mL of 80% nitric acid, 18 mL glacial acetic acid, and 20 g mercuric chloride; Bagenal and Braum 1978). However, ovarian tissue hardens in formalin, making oocyte separation difficult, and Gilson’s fluid can cause oocyte shrinkage (15%; DeMartini and Fountain 1981) and degeneration of hydrated oocytes (Brown-Peterson et al. 1988). Because of these problems and concerns about mercury toxicity (West 1990), physical separation of oocytes prior to fixation and preservation in 2% buffered formalin was recommended by Lowerre-Barbieri and Barbieri (1993). Fish eggs collected in plankton samples are often fixed and preserved in buffered formalin (Ahlstrom 1976; Smith and Richardson 1977; Checkley et al. 2000), but use of buffered formalin solutions sometimes results in inadequate preservation (Ahlstrom 1976; Markle 1984; Gates et al. 1987), and un-buffered 4–7% formalin has been recommended (Markle 1984; Klinger and Van Den Avyle 1993).

For long-term preservation of fish larvae, 3–5% formaldehyde buffered with 1% sodium acetate should result in limited shrinkage and good pigment preservation without decalcification (Smith and Richardson 1977; Tucker and Chester 1984). Alternative buffers include sodium borate (borax; Ahlstrom 1976), calcium carbonate (marble chips or limestone powder; Steedman 1976), and sodium phosphate (1.8 g sodium phosphate monobasic and 1.8 g anhydrous sodium phosphate dibasic [0.013 M, pH 6.8] in 1 L of 5% formalin; Markle 1984). The acidity of formalin-based solutions can increase over time because of production of formic acid from oxidation (Steedman 1976). Buffering to pH 7.0–7.5 (Tucker and Chester 1984) will prevent bone (including otolith) decalcification and demineralization (Taylor 1977). However, high pH (>8.0) may increase larval transparency (clearing), de-pigmentation (specifically sodium borate; Taylor 1977), formation of calcium carbonate crystals (Tucker and Chester 1984), and precipitation of sodium phosphate on specimens (Markle 1984). Concerns over the carcinogenic nature of aldehydes (Smith 1992) have led to greater use of less toxic, but more expensive, propylene-glycol-based preservatives (e.g., Carosafe, Carolina Biological Supply Company, and Formalternate, Flinn Scientific), at least for short-term preservation and storage after fixation.

Freezing or alcohol-based solutions are preferred for storing fish larvae for genetic (e.g., Pegg et al. 2006; Vigliola et al. 2007), stable isotope (e.g., Pepin and Dower 2007), and age and growth studies (e.g., Dower et al. 2009), although freezing can distort length–mass relationships, increase difficulties with morphological measurements, and introduce errors into morphology-based analyses such as length–weight relationships (Sayers 1987; Armstrong and Stewart 1997). For genetic studies, ichthyoplankton should be frozen rapidly in liquid nitrogen and stored at −76°C or below to retain the biochemical properties of proteins and DNA. Long-term freezing may cause shrinkage (but typically less than formalin-based preservation), cellular damage (Halliday and Rosco 1969; Jones and Green 1977), and disproportionately high loss of nitrogen compared with chemical fixation (Williams and Robins 1982). Specimens frozen to avoid shrinkage, clearing, or decalcification should be processed as soon as possible.
Color preservation. Antioxidants have been used to preserve color in market fish (Wasson et al. 1991) but have not been extensively tested as color preservatives for specimen identification (but see Gerrick [1968] regarding adult fish and Ahlstrom [1976] for larvae). Acidic and neutral formalin-based preservatives generally preserve brown and black melanins well without the use of antioxidants, and because color has not historically been an important character in the identification of larval fish or zooplankton, the addition of antioxidants is unnecessary in most cases. However, in some collections, natural color preservation is required (e.g., red hues in larval tunas), and the addition of 0.2–0.4% solutions of IONOL CP-40 (40% butylated hydroxyl-toluene) has been successful in preserving color (Berry and Richards 1973; Scotton et al. 1973). Examination of specimens immediately after collection is the best approach if color is an important attribute (Ahlstrom 1976).

9.4.3 Zooplankton

There are many types of fixatives for marine zooplankton (Steedman 1976), but a seawater solution of 4% formaldehyde buffered with sodium borate and strontium chloride (Sameoto et al. 2000) or sodium tetraborate (Postel et al. 2000) should suffice for most applications. In freshwater systems, ethanol is recommended as a fixative for freshwater cladocerans (95%; Dodson et al. 2010), copepods (70%; Reid and Williamson 2010), and rotifers (30–50%; Wallace and Snell 2010), although it has been suggested that *Moina* spp. (and perhaps other taxa) should be narcotized first (Smith 2001). If phyllopodous branchiopods are believed to be present, samples should be fixed in 4% formaldehyde to prevent distortion of antennal characteristics (Beladjal and Mertens 1999; Johnston and Cunjak 1999). All of these fixatives may cause morphological distortion (De Bernardi 1984) but may better prevent loss of mass and maintain nucleic structure (Kimmerer and McKinnon 1986). If morphological distortion and volume loss (Ahlstrom and Thrailkill 1963) are concerns, freezing zooplankton at −18°C or on dry ice may reduce physical damage (Omori 1978) and facilitate direct biomass measurements (Postel et al. 2000).

Generally, 70–80% ethanol is recommended as a long-term preservative for marine (Steedman 1976) and freshwater (Smith 2001) zooplankton. Plankton may also be stored in a 4% sucrose–formalin solution (Haney and Hall 1973), as the high sugar concentrations prevent microbiologically induced decomposition. Addition of glycerin to specimen containers may help prevent specimen damage if container seals fail over time (Dodson et al. 2010). Fresh specimens should be used for stable isotope research whenever possible, and guts should be evacuated prior to freezing or fresh-tissue analyses (Feuchtmayr and Gray 2003). Freezing is typically used for zooplankton storage in genetic studies (e.g., liquid nitrogen; Vanoverbeke and De Meester 1997), although techniques for mitochondrial DNA (mtDNA) analyses of samples stored for extended periods in buffered formalin have been developed (e.g., Bucklin and Allen 2004).

9.5 SAMPLE PROCESSING

Fixed, frozen, or otherwise preserved specimens are typically returned to a laboratory for sorting, counting, identification, measurement, and other analyses (e.g., age determination, gut analysis, or genetic analyses). In some cases, shipboard processing can enhance subsequent laboratory processing (Postel et al. 2000). Filter columns and sieves can be used to sort live specimens into size-classes, which can be helpful in the separation of taxonomic groups or developmental stages or both (for zooplankton, see Seda and Dostálková [1996]), although sieving may cause damage to collected organisms. Illustration of larval fish, egg, and zooplankton characteristics can be an
important part of taxonomic studies, and use of photomicroscopy, real-time video microscopy, and digital imaging technology can greatly improve illustrative efforts. Digital technology offers many advantages in image capture and magnification, as well as rapid dissemination of imagery to other taxonomic researchers. It is important that specimens and all data associated with their collection and processing (e.g., date, location, collection personnel, fixative, and physicochemical data) ultimately be deposited for long-term curation with an appropriate teaching or museum collection (Lavenberg et al. 1984), where they will be available for voucher, taxonomic reference, and specimen-specific studies.

9.5.1 Subsampling

Subsampling is typically not recommended for fish eggs and larvae, although it is commonly used for zooplankton and may be necessary when fish larvae and egg densities are very high (e.g., use of the Folsom plankton splitter [see below]; Lewis and Garriott 1971; Smith and Richardson 1977; Paolucci et al. 2007). Time saved by subsampling may be outweighed by potential specimen damage and bias introduced by the subsampling method (Griffiths et al. 1976). Subsamples also may not adequately represent the presence and abundance of rare taxa and developmental stages in a sample (Sell and Evans 1982).

Zooplankton collected in the field is typically concentrated in 100–500 mL bottles for fixation, and high numbers of collected organisms (often several hundred per liter) may make examination of the entire sample impractical. Subsampling can be accomplished by several methods (see Edmondson 1971 for a good discussion of zooplankton enumeration methods), one of which involves pouring a sample that has been well mixed by vigorous stirring, repeated pouring between beakers, or agitation with an electric stirrer or air hose (Kaller and Hartman 2004) into a Folsom plankton splitter (Sell and Evans 1982) or Motodo plankton splitter (Snelgrove et al. 2008). These devices divide the sample into two equal subsamples (coefficient of variation [CV] for the Folsom splitter estimated to be 5–18%; van Guelpen et al. 1982), and the process can be repeated several times until a subsample with a reasonable number of organisms is produced. Plankton are identified and counted in these subsamples, and abundances in the original sample are then calculated by multiplication (e.g., times 2 for half a sample). Results of studies by Sell and Evans (1982) indicated that examination of one to three subsamples per sample (the sample was split 10 times) was adequate to quantify plankton in the original sample. An alternative method involves a predetermined number of aliquots that are removed from the mixed sample with a Hensen–Stempel pipette (CV 7–9%) or bulb pipette (CV 14–15%; van Guelpen et al. 1982), with specimens completely enumerated in each aliquot. Abundance in the original sample is calculated by summing the volume of the aliquots, dividing the original sample volume by the total aliquot volume, and multiplying this value times the total number of organisms in the aliquots. For example, suppose five 1-mL aliquots that are extracted from a well-mixed sample of 200 mL yield 8, 11, 15, 9, and 12 Daphnia. The estimated number of Daphnia in the original sample would be 200/5 × 55 = 2,200. To calculate plankton densities encountered in the water body at the time of sampling (e.g., number/m³), the estimate would be divided by the volume of water sampled by the gear.

Counting chambers such as the Sedgewick–Rafter cell or Bogorov tray have been used for identification and abundance estimation of fish eggs and zooplankton, but counting errors are common when using these devices and can significantly affect abundance estimates (Lund et al. 1958; Cassie 1971). Citing these studies and practical experience, Postel et al. (2000) indicated
precision of ±20% was acceptable and could be attained by counting 100 of each of the most common taxa; abundances of taxa with counts less than 100 should not be estimated.

Variability in data associated with subsampling (Elliott 1977; Griffiths et al. 1984) should be evaluated by comparisons among groups of subsamples and an entirely processed sample. For example, one could remove five aliquots from a mixed zooplankton sample, count the animals in the aliquots, return these five subsamples to the sample, and repeat the process several times, followed by complete enumeration of the sample. The CV among subsample group totals would provide information on precision, and the ratio of the estimated number of organisms in the subsample groups to the total count would provide estimates of accuracy. From the Daphnia example, the CV among the five subsamples is $2.45/11 = 22.2\%$. Suppose additional subsampling trials yield estimates of 2,254, 2,307, and 2,189 Daphnia in the sample, and total enumeration yields 2,314 individuals. In this case, the subsamples would have yielded accuracies ranging from 94.6 to 99.7%, or an average of 96.7 ± 2.0% (SD). Reported CVs of subsampling devices range from 5 to 30% (Kott 1953; van Guelpen et al. 1982; Sell and Evans 1982). However, subsamples are not individual experimental units and therefore are unsuitable for statistical analyses of questions regarding plankton abundance. Rather, subsamples should be used only to describe experimental units, which are the smallest units on which a treatment is applied or an effect is measured (Hurlbert 1984; Heffner et al. 1996; Chapter 2), usually the extrapolated density of plankton in a tow.

### 9.5.2 Sorting

Sorting target organisms from undesired organisms and debris is usually necessary because most samples of fish eggs, larvae, and zooplankton also contain other plankton. The time necessary for sorting will depend on the size of the sample and the size of the target organisms. Care should be taken in selecting sorting protocols, as substantial error (missed organisms) may occur during sorting that can influence results (Scotton et al. 1973; van Guelpen et al. 1982; Ettinger 1984; Haase et al. 2004, 2006). Accuracy should be checked by careful re-inspection of the unwanted portions of several sorted samples before they are discarded.

During sorting, larval fish residence times in water should be minimized to avoid reversing the effects of formalin fixation (Taylor 1977), and researchers should wear gloves and return specimens to a dilute formalin preservative solution as soon as possible or sort larvae in dilute formalin if the work area is adequately exhaust ventilated. Alcohol-fixed organisms should be sorted in the preservative to avoid strong, potentially damaging osmotic effects on internal structure of organisms placed in water. Avoid exposing organisms to extended periods of bright light and warm temperatures, which can affect specimen quality and eventually cause melanophore pigments in larval fish to fade. Eggs, larvae, and zooplankton are fragile and easily damaged by hard surfaces, rigid tools, and rough handling; therefore, specimens should be handled gently with pipettes, wire loop probes, and flexible forceps. Commonly, samples are sorted in gridded petri dishes, Sedgewick–Rafter counting cells, large glass culture or baking dishes placed on a high-contrast background, illuminated black or white enamel trays, or side-lit sorting chambers (Dorr 1974) under low-power magnification with dissecting microscopes or illuminated magnifying lamps. Dissecting and compound microscopes (depending on organism size) are typically used to identify ichthyoplankton and zooplankton. Polarized light can aid in plankton counting and identification, and transmitted polarized light may be necessary to discern larval fish myomeres (serial muscle segments of the body; section 9.6.4). Although not amenable for all plankton stud-
ies, the application of automated or manual digital imagery and associated computer software to plankton sorting, identification, and measurement continues to grow and offers the potential advantages of reduced specimen damage and identification errors (Benfield et al. 2007). Several computer software packages are available for plankton identification (e.g., Visual Plankton written for MatLab [Davis et al. 2005]; PISCES [Luo et al. 2005]; Zooprocess Plankton Identifier [www.zooscan.com]). Biological stains can reduce sorting time and increase sorting efficiency of ichthyoplankton and zooplankton samples (Mason and Yevich 1967; Mitterer and Pearson 1977; Fleming and Coughlan 1978), but stains may obscure myomeres and other morphological features used to identify fish larvae. Rose bengal has been used to aid in sorting of larval fish samples without loss of identification accuracy (Talbot and Able 1984; Feyer 2004; Overton and Rulifson 2007), and rose bengal, eosin and biebrich scarlet (1:1; Klinger and Van Den Avyle 1993), phloxine B (Mason and Yevich 1967), and Lugol’s iodine counterstained with chlorazol (Williams and Williams 1974) are effective stains of fish eggs. Although not commonly used, zooplankton samples can be stained with eosin Y (Edmondson 1971) and neutral red (Fleming and Coughlan 1978).

9.6 PLANKTON IDENTIFICATION

Most of the world’s diversity of fish eggs, larvae, and zooplankton are poorly described, and discrimination of species continues to be problematic for many morphologically variable groups (e.g., marine dinoflagellates; Culverhouse et al. 2003). Identification to species, or even genus or family, may be impossible for many planktonic organisms given the current state of taxonomic knowledge. As a consequence, taxonomic assignments should be made with great care and based on considerable evidence. When in doubt, use the lowest level of taxonomic resolution that can be assigned with confidence (e.g., a family assignment rather than genus). Taxonomic information can be obtained from general and regional keys or manuals (Tables 9.2 and 9.3), as well as comparative and individual descriptions in the literature, reference or voucher collections, and taxonomic experts. Identification protocols should be thoroughly described when results are published.

9.6.1 Fish Egg Development

Egg development is a dynamic process typically assumed to encompass the time from ovulation until hatching. Fish egg structure consists of an outer membrane (chorion), perivitelline space, an inner egg membrane (in some fishes), and yolk (Ahlstrom and Moser 1980; Kendall et al. 1984; Figure 9.10). Most fishes are oviparous, which involves releasing eggs to the external environment after ovulation, followed by fertilization. Upon fertilization, eggs undergo changes within minutes in structure, color, and function (egg activation) that prevent multiple fertilization (polyspermy), harden the chorion (water hardening; Redding and Patino 1993), and begin embryonic development. Cell division in fish eggs is most commonly meroblastic (partial cleavage), although cleavage may be holoblastic (total cleavage, e.g., lampreys) or intermediate (e.g., South American lungfish, sturgeons, gars, and bowfin; Blaxter 1969; Lagler et al. 1977). Crim and Glebe (1990), Redding and Patino (1993), Yaron and Levavi-Sivan (2005), and Rocha et al. (2007) provide general reviews of fish reproduction including gonad maturation, gonosomatic indices, and fecundity estimates.

Descriptions of the stages of egg and embryo development can also aid in their identification. A simple categorization includes early development (from fertilization to closure of the blastopore), middle development (from closure of the blastopore to tail bud separation), and
Table 9.2  Selected taxonomic guides and keys for the identification of freshwater and marine fish eggs and larvae. Many manuals are illustrated and include regional notes on the distribution and ecology of adult spawning, eggs, and larvae.

<table>
<thead>
<tr>
<th>Author(s), date</th>
<th>Region</th>
<th>Coverage, comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auer 1982</td>
<td>Great Lakes</td>
<td>148 species accounts; keys</td>
</tr>
<tr>
<td>Conrow and Zale 1985</td>
<td>Florida</td>
<td>18 species accounts</td>
</tr>
<tr>
<td>Drewry 1979</td>
<td>Great Lakes</td>
<td>Punch-card key to yolk sac larvae</td>
</tr>
<tr>
<td>Fish 1932</td>
<td>Lake Erie</td>
<td>62 species accounts; several misidentifications</td>
</tr>
<tr>
<td>Hogue et al. 1976</td>
<td>Tennessee River</td>
<td>32 species descriptions; photographs and keys</td>
</tr>
<tr>
<td>Holland-Bartels et al. 1990</td>
<td>Upper Mississippi River</td>
<td>19 illustrated families; 63 unillustrated species</td>
</tr>
<tr>
<td>Kay et al. 1994</td>
<td>Ohio River basin</td>
<td>21 species accounts, Catostomidae</td>
</tr>
<tr>
<td>May and Gasaway 1967</td>
<td>Oklahoma</td>
<td>18 species accounts; photographs and key</td>
</tr>
<tr>
<td>McGowan 1984</td>
<td>South Carolina</td>
<td>11 families, 18 species</td>
</tr>
<tr>
<td>McGowan 1988</td>
<td>North Carolina</td>
<td>3 Piedmont impoundments</td>
</tr>
<tr>
<td>Nakatani et al. 2001</td>
<td>Paraná, Brazil</td>
<td>22 families, 62 species accounts, keys (in Portuguese)</td>
</tr>
<tr>
<td>Ponton and Mérigoux 2001</td>
<td>River Sinnamary, French Guiana</td>
<td>22 families, 77 species (in English)</td>
</tr>
<tr>
<td>Simon and Wallus 2004</td>
<td>Ohio River basin</td>
<td>22 species accounts, Ictaluridae; keys and comparative tables</td>
</tr>
<tr>
<td>Simon and Wallus 2006</td>
<td>Ohio River basin</td>
<td>85 species accounts, Percidae; keys and comparative tables</td>
</tr>
<tr>
<td>Snyder 1981</td>
<td>Upper Colorado River basin, Colorado</td>
<td>19 species accounts, Cyprinidae and Catostomidae; keys and comparative table</td>
</tr>
<tr>
<td>Snyder et al. 2004</td>
<td>Upper Colorado River basin, Arizona, Colorado, New Mexico, Utah, and Wyoming</td>
<td>7 species accounts, Catostomidae; comparative tables, computer-interactive key</td>
</tr>
<tr>
<td>Snyder et al. 2005</td>
<td>Gila River basin, Arizona</td>
<td>14 species accounts, Cyprinidae and Catostomidae; comparative tables, computer-interactive keys</td>
</tr>
<tr>
<td>Sturm 1988</td>
<td>Alaska freshwater</td>
<td>9 families, 21 species accounts</td>
</tr>
<tr>
<td>Wallus et al. 1990</td>
<td>Ohio River basin</td>
<td>24 species accounts, Acipenseridae through Esocidae; keys and comparative tables</td>
</tr>
<tr>
<td>Wallus et al. 2006</td>
<td>Ohio River basin</td>
<td>25 species accounts, Aphredoderidae through Cottidae, Moronidae, and Sciaenidae</td>
</tr>
<tr>
<td>Wallus and Simon 2008</td>
<td>Ohio River basin</td>
<td>21 species accounts, Elassomatidae and Centrarchidae; keys and comparative tables</td>
</tr>
<tr>
<td>Author(s), date</td>
<td>Region</td>
<td>Coverage, comments</td>
</tr>
<tr>
<td>----------------</td>
<td>--------</td>
<td>--------------------</td>
</tr>
<tr>
<td><strong>North American Estuarine and Coastal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colton and Marak 1969</td>
<td>Northeast coast</td>
<td>27 species accounts</td>
</tr>
<tr>
<td>Elliot and Jimenez 1981</td>
<td>Beverly-Salem Harbor, Massachusetts</td>
<td>47 species accounts</td>
</tr>
<tr>
<td>Garrison and Miller 1982</td>
<td>Puget Sound, Washington</td>
<td>124 species accounts</td>
</tr>
<tr>
<td>Lipson and Moran 1974</td>
<td>Potomac River estuary</td>
<td>88 species accounts; keys</td>
</tr>
<tr>
<td>Mansueti and Hardy 1967</td>
<td>Chesapeake Bay</td>
<td>45 species accounts; Acipenseridae through Ictaluridae</td>
</tr>
<tr>
<td>Scotton et al. 1973</td>
<td>Delaware Bay</td>
<td>56 species accounts</td>
</tr>
<tr>
<td>Wang 1981</td>
<td>Sacramento–San Joaquin Estuary, California</td>
<td>74 species accounts; comparative tables</td>
</tr>
<tr>
<td>Wang 1986</td>
<td>Sacramento–San Joaquin Estuary and adjacent waters</td>
<td>43 families, 125 species accounts; comparative tables</td>
</tr>
<tr>
<td>Wang and Kernehan 1979</td>
<td>Delaware estuaries</td>
<td>50 families, 113 species accounts, keys</td>
</tr>
<tr>
<td><strong>Atlantic Ocean</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ditty and Shaw 1994</td>
<td>Western central Atlantic</td>
<td>21 genera, 55 species, Sciaenidae</td>
</tr>
<tr>
<td>Fahay 1983</td>
<td>Western North Atlantic</td>
<td>290 species accounts</td>
</tr>
<tr>
<td>Fahay 2007</td>
<td>Western North Atlantic</td>
<td>760 species from 196 families</td>
</tr>
<tr>
<td>Farooqi et al. 1995</td>
<td>Western central Atlantic</td>
<td>7 genera, 28 species, Engraulidae</td>
</tr>
<tr>
<td>Hardy et al. 1978</td>
<td>Mid-Atlantic Bight</td>
<td>278 species accounts; includes tidal freshwater zones</td>
</tr>
<tr>
<td>Munk and Nielsen 2005</td>
<td>North Sea</td>
<td>96 species accounts</td>
</tr>
<tr>
<td>Olivar and Fortuno 1991</td>
<td>Southeast Atlantic</td>
<td>127 taxonomic accounts; illustrations</td>
</tr>
<tr>
<td>Richards 2006</td>
<td>West central Atlantic</td>
<td>213 families, 2,080 species accounts</td>
</tr>
<tr>
<td>Russell 1976</td>
<td>British Isles marine waters</td>
<td>40 families; taxonomic characters and methods</td>
</tr>
<tr>
<td><strong>Pacific and Indian Oceans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leis and Carson-Ewart 2004</td>
<td>Indo-Pacific coastal waters</td>
<td>124 family accounts</td>
</tr>
<tr>
<td>Leis and Rennis 1983</td>
<td>Indo-Pacific coral reefs</td>
<td>49 family accounts</td>
</tr>
<tr>
<td>Leis and Trnoki 1989</td>
<td>Indo-Pacific shorelines</td>
<td>54 family accounts</td>
</tr>
<tr>
<td>Matarese et al. 1989</td>
<td>Northeast Pacific</td>
<td>232 species accounts; keys</td>
</tr>
<tr>
<td>Miller et al. 1979</td>
<td>Hawaiian Islands</td>
<td>30 families</td>
</tr>
<tr>
<td>Moser 1996</td>
<td>California current</td>
<td>Full descriptions of 141 families, 467 species; partial descriptions of 17 families, 119 species; comparative tables</td>
</tr>
<tr>
<td>Neira et al. 1996</td>
<td>Australia coastal marine and freshwater</td>
<td>50 families, 124 species accounts; 93 new accounts</td>
</tr>
</tbody>
</table>
### Table 9.2  Continued

<table>
<thead>
<tr>
<th>Author(s), date</th>
<th>Region</th>
<th>Coverage, comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nishikawa and Rimmer 1987</td>
<td>Indian Ocean, northwestern Australia</td>
<td>21 scombroid fishes; descriptions, notes, and keys</td>
</tr>
<tr>
<td>Ozawa 1986</td>
<td>Western North Pacific</td>
<td>15 families, 159 species</td>
</tr>
</tbody>
</table>

**Europe**

| Pinder 2001 | British Isles | Freshwater, 6 families, 26 species; keys |

### Table 9.3  Selected taxonomic guides and keys for identification of freshwater (North and Central America) and marine zooplankton, with notes on coverage and regional specificity.

<table>
<thead>
<tr>
<th>Author(s) or editors, date</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Freshwater</strong></td>
<td></td>
</tr>
<tr>
<td>Balcer et al. 1984</td>
<td>Great Lakes</td>
</tr>
<tr>
<td>Collado et al. 1984</td>
<td>Costa Rica copepods</td>
</tr>
<tr>
<td>Fernando 2002</td>
<td>Tropical freshwater</td>
</tr>
<tr>
<td>Green 1997</td>
<td>British Columbia cladocerans</td>
</tr>
<tr>
<td>Hudson et al. 1998</td>
<td>Great Lakes copepods</td>
</tr>
<tr>
<td>Hudson and Lesko 2003</td>
<td>Great Lakes copepods</td>
</tr>
<tr>
<td>Hudson et al. 2003</td>
<td>Great Lakes copepods</td>
</tr>
<tr>
<td>Sandercook and Scudder 1996</td>
<td>British Columbia calanoid copepods</td>
</tr>
<tr>
<td>Smith 2001</td>
<td>North American freshwaters</td>
</tr>
<tr>
<td>Smith and Fernando 1978</td>
<td>Ontario cyclopoid copepods</td>
</tr>
<tr>
<td>Stemberger 1979</td>
<td>Great Lakes rotifers</td>
</tr>
<tr>
<td>Thorp and Covich 2010</td>
<td>North American freshwaters</td>
</tr>
<tr>
<td>UNH Center for Freshwater Biology</td>
<td>Northeastern United States</td>
</tr>
<tr>
<td><strong>Marine</strong></td>
<td></td>
</tr>
<tr>
<td>Boltovskoy 1981</td>
<td>Southwest Atlantic (in Spanish)</td>
</tr>
<tr>
<td>Boltovskoy 1999</td>
<td>South Atlantic</td>
</tr>
<tr>
<td>ICES 1939</td>
<td>North Atlantic (in French)</td>
</tr>
<tr>
<td>Johnson and Allen 2005</td>
<td>Atlantic and Gulf of Mexico coasts</td>
</tr>
<tr>
<td>Newell and Newell 1979</td>
<td>General marine</td>
</tr>
<tr>
<td>Reidl 1983</td>
<td>Mediterranean (in German)</td>
</tr>
<tr>
<td>Schram 1986</td>
<td>Marine crustaceans</td>
</tr>
<tr>
<td>Smith and Johnson 1996</td>
<td>Coastal waters</td>
</tr>
<tr>
<td>Todd et al. 1996</td>
<td>Coastal waters</td>
</tr>
<tr>
<td>Trégouboff and Rose 1957</td>
<td>Mediterranean (in French)</td>
</tr>
<tr>
<td>Wickstead 1965</td>
<td>Tropical marine</td>
</tr>
<tr>
<td>Yamaji 1971</td>
<td>Japanese coastal waters</td>
</tr>
<tr>
<td>Young et al. 2006</td>
<td>Marine invertebrates</td>
</tr>
</tbody>
</table>
late development (from tail bud separation to hatching; Ahlstrom and Ball [1954]; Matarese and Sandknop [1984]). A more detailed egg development description includes (1) early cleavage: 1–64 cells; (2) morula: blastomeres that form a cluster of cells; (3) blastula: formation of the blastocoels; (4) gastrula: differentiation of ectoderm, mesoderm, and endoderm; (5) early embryo: formation of the embryonic axis; (6) tail bud: prominent caudal bulge and cephalic development; (7) tail free: separation of the tail from the yolk; and (8) late embryo: developing characteristics of the hatching stage (Mansueti and Hardy 1967).
9.6.2 Fish Egg Identification

Egg size, shape, and structure vary considerably among fishes and may be useful for identification. Overall, fish eggs average 1 mm in diameter, with a range of about 0.3 mm in the cyprinid *Paedocypris* to 90 mm in the coelacanth (Balon 1991; Kottelat et al. 2006). Although most commonly translucent, some fish eggs are darkly colored (e.g., paddlefishes, sturgeons, and gars). Eggs can be buoyant (pelagic species) or nonbuoyant (demersal species), adhesive (e.g., pikes and pickerels) or nonadhesive (e.g., walleye and sauger) and are sometimes modified with structures that aid attachment or flotation. Although typically spherical, fish eggs may also be ovoid or irregularly shaped, and oil globules, when present, can vary in number, size, color, and position. The yolk may have a characteristic texture or segmentation, color, and circulatory structure (later embryonic development), and the chorion may vary in surface topography, ornamentation, thickness, color, coatings, attachment structures, and micropyle size. The width of the perivitelline space and the presence and location of the inner egg membrane may vary as well. These characteristics are used for identification in combination with embryonic characters, collection information (e.g., location, water temperature, season, and collection gear), and mode of reproduction (Hempel 1979; Newell and Newell 1979; Ahlstrom and Moser 1980; Matarese and Sandknop 1984; Balon 1985; Blaxter 1988; Table 9.2). Because eggs can be particularly difficult to identify, researchers have explored numerous techniques to improve identification accuracy including mechanical aids (e.g., scanning electron microscopy; Riehl and Kock 1989) and biochemical techniques such as immunodiffusion and immunofluorescence (Johnson et al. 1975), molecular degradation (Valcarce et al. 1991), gas chromatography (Knutsen et al. 1985), isoelectric focusing (Mork et al. 1983), protein electrophoresis (Scobbie and Mackie 1990), and mtDNA analyses (Graves et al. 1990). Polymerase chain reaction (PCR) methods have also been used for egg identification, either in combination with electrophoretic techniques (restricted fragment length polymorphism [RFLP]; Rocha-Olivares 1998) or RFLP-HPLC (RFLP used in conjunction with high performance liquid chromatography; Horstkotte and Rehbein [2003]).

9.6.3 Larval Fish Development

A number of terminologies (Snyder 1979; Kendall et al. 1984; Snyder and Holt 1984; Balon 1985; Blaxter 1988) exist to describe developmental intervals of fish larvae. Each of these is problematic because attempting to categorize dynamic and often species-specific processes into a static classification scheme is inherently difficult. Generally, researchers define the “embryonic period” as development from fertilization to hatching (but see Balon 1984), the “juvenile period” from the acquisition of an adult body form to sexual maturation, and the “larval period” as the interval between embryo and juvenile. Although “fry” is sometimes used as an alternative term for larvae, mostly in fish culture, there is little agreement on a precise definition for this term. Three commonly accepted terminologies are currently used to categorize the phases of larval fish development.

1. Mansueti and Hardy (1967) and Hardy et al. (1978) described three phases of larval fish development based on the presence or absence of yolk and fin ray development.

   *Yolk-sac larvae:* phase between hatching and yolk absorption.

   *Larvae:* phase between yolk absorption and the acquisition of adult fin ray complement.

   *Prefrereural or transitional:* intermediate phase between larval and juvenile forms of certain species that begins with the acquisition of the minimum adult fin ray complement and terminates in a more adult-like juvenile form.
2. Ahlstrom et al. (1976) used changes in the homocercal caudal fin in their terminology.

*Preflexion larvae:* phase between hatching and upward flexing of the tip of the notochord or appearance of the first caudal rays.

*Flexion larvae:* phase characterized by the upward flexion of the notochord terminating with the formation of all principal caudal rays and the first appearance of secondary caudal rays.

*Postflexion larvae:* phase beginning after upward flexion of tip of the notochord and terminating with a complete complement of fin rays. For some species, prejuvenile or transitional phases are applied.


*Protolarvae:* phase between hatching and appearance of the first median fin ray or spine (dorsal, anal, or caudal fins).

*Mesolarvae:* phase beginning with the appearance of the first median fin ray or spine and terminating with acquisition of the pelvic fins or fin buds and a full complement of principal soft fin rays in the median fins.

*Metalarvae:* phase beginning with acquisition of pelvic fins or fin buds and a full complement of principal soft fin rays in the median fins and terminating with the loss of all fin folds and acquisition of the adult complement of spines and rays (including some ray segmentation) in all fins.

Each of these terminologies has been used successfully, and none currently dominates the early life history literature, probably because of historic inertia as well as the broad array of topics covered in ichthyoplankton research (e.g., ontogeny, taxonomy, physiology, and ecology). Any terminology adopted to describe larval fish development should be inclusive of the diversity of forms, have some morphological or functional significance in the life history of the fish, and have observable and well-defined endpoints for each phase (Kendall et al. 1984). Although Snyder’s (1976, 1981) terminology was traditionally more common in studies of freshwater larval fishes in North America, several marine researchers (Kendall et al. 1984; Blaxter 1988) have advocated the use of the Ahlstrom et al. (1976) terminology because of the functional importance of caudal fin development. Although all three schemes are currently being used, entries in LarvalBase (www.larvalbase.org) do not frequently use the terminology of Snyder (1976, 1981). Ultimately, a combination of the terminologies (e.g., postflexion mesolarvae with yolk, yolk sac mesolarvae; Snyder and Holt 1984), as discussed by Snyder and Muth (1990) and Snyder et al. (2004, 2005), may prove most useful for standardization of terminology and definitions.

9.6.4. Larval Fish Identification

Meristic and morphometric characteristics, pigmentation patterns, larval shape, size, and osteological development are used to identify larval fish (Kendall et al. 1984; Figure 9.10). Adult morphological characters may be useful for the identification of late larvae but not earlier phases because of developmentally related structural differences. Some larvae possess specialized structures that are unique to the larval period and useful for identification. Examples include eye stalks (e.g., black dragonfish; Weihs and Moser 1981), elongated dorsal fins (e.g., flounder, horned whiff; Tucker 1982), adhesive organs (e.g., bowfin; Simon 1990), unique spines (e.g., the head spines of rockfishes in the genus *Sebastes*; Kendall 1991), trailing guts (e.g., stomiid dragonfishes; Kawaguchi and Moser 1984), and photophores (e.g., myctophid lanternfishes; Moser and Ahl-
strom 1974). More frequently, myomere counts and the size, shape, and position of the gut, air bladder, yolk sac, oil globules, mouth, fin folds, and fins are used. Melanophore pigmentation patterns may be particularly useful in species identification (Berry and Richards 1973; Snyder 1981; Kendall et al. 1984). Generally, taxonomic characteristics vary throughout the larval period, requiring that most meristic, morphometric, or other characters be related to specific sizes or developmental stages.

9.6.4.1 Myomere Counts

Myomeres, which are chevron-shaped serial segments of body musculature separated by connective tissue (myosepta), are conspicuous morphological features that approximate the number and position of vertebrae (typically, one more than the number of vertebrae), although vertebral counts are less variable (Snyder 1976; Fuiman 1982). Because of their relative consistency throughout the larval period, total and partial myomere counts are useful identification characters. Total myomere counts include all myomeres from the first myomere, posterior to the occiput, to the urostylar myomere (Fuiman 1982). Preanal myomere counts include all myomeres anterior to the posterior margin of the anus and include myomeres transected by an imaginary vertical line from that point. Postanal myomeres are those entirely posterior to this imaginary line (Siefert 1969). Other partial myomere counts may be useful to reference the location of important structural features.

9.6.4.2 Morphometric Characters

Morphometric characteristics used for taxonomic purposes generally describe body form features such as body depth or eye width (Figure 9.10). Many morphometric characters are allometric (i.e., larval fish change shape systematically as they grow) and are often reported as ratios (proportions or percentages) for comparative purposes to account for the influence of body size on character size (e.g., head length to body length ratio). Ratios have inherent statistical problems (e.g., inflated standard errors, nonparametric frequency distributions, and potentially erroneous correlations; Atchley et al. 1976), which have led to the development of several regression methods to avoid these problems (Strauss and Bond 1990). Generalized linear model theory (Nelder and Wedderburn 1972; McLean et al. 1991), desktop computing power, and statistical software offer viable options for handling ratio data (Agresti 2007) in comparative studies of larval fishes.

Although more methodologically complex and time-consuming, truss network analysis (Box 9.2) of fish shape offers an alternative to ratio-based morphometric analyses for identification and has been used to characterize the shape of adult (Strauss and Bookstein 1982; Strauss and Bond 1990; Bookstein 1991) and larval fishes (Strauss and Fuiman 1985; Fulford and Rutherford 2000). Truss analysis is a multivariate statistical technique that quantifies the shape (oblique, longitudinal, and vertical) of an organism with distance measurements among anatomical landmarks (see Douglas 1993 for application of video imaging technology to truss analysis). It is based on the assumption that anatomical landmarks are homologous among species. Despite a limited availability of landmarks on larval fish, several prominent features (e.g., snout tip, bone articulations, and tip of urostyle) can be located to divide specimens into functional units. Landmarks are selected to form contiguous quadrilaterals (anterior to posterior), with the landmarks forming the boundary of each quadrangle (truss cell). Within each truss cell, six pairwise measurements are made. Ordination methods (e.g., principal component, sheared component, and discriminant function analyses) commonly are used to describe size and shape differences based on morphometric sets developed from the truss protocol (Humphries et al. 1981; Bookstein et al. 1985;
Box 9.2 Morphometric Analyses of Larval Shape

A number of morphometric approaches are used to identify fishes and fish stocks (Humphries et al. 1981; Strauss and Bookstein 1982; Cadrin and Friedland 1999) and to assess fish condition (Fitzgerald et al. 2002). Landmark-based analysis evolved from traditional methods (Cadrin and Friedland 1999) to increase discrimination among groups by increasing the number of measurement points (landmarks) and the number of distances measured between them (Humphries et al. 1981; Strauss and Bookstein 1982). Landmark analysis using multivariate statistical methods and advanced digital-imaging technology to increase measurement precision and control magnification now offers high-resolution discrimination of fish species and stocks (Cadrin 2000). This method may be particularly appropriate for larval fishes, which are often difficult to identify. Images of live fish can be used (Douglas 1993), which offers a particularly useful tool for discrimination of uncommon, threatened, or endangered species.

Distances between landmarks are measured in standard units. The number of landmarks is determined by the researcher based on specimen architecture; 10 to 16 landmarks are typically used. The number of fish collected should be 3.5 to 8.0 times the number distances measured (Kocovsky et al. 2009), which is lower than for other ordination guidelines (e.g., up to 20:1 in Stevens 2002) and is fortunate when specimens are rare or uncommon. Landmarks are commonly measured with digitally equipped dissecting microscopes, although video cameras with image capture are acceptable alternatives. After an image is captured, measurements can be made with software that may be proprietary to the microscope or camera (e.g., NIS-ELEMENTS for Nikon digital cameras) or geographical information system software packages (e.g., ARCMAP 9.2, Esri, Inc.) adapted for measuring image distances. Subsequent data analysis depends on the research question and type of measurements. If the research question is exploratory (i.e., multiple groups are suspected but not known), multi-group principal component analysis (PCA) should be performed on direct landmark measurements (e.g., Cadrin and Silva 2005). If two or more morphologically similar groups are believed to be present based on prior knowledge, experience, or distribution, discriminant analysis (e.g., linear or canonical discriminant function analysis) should be performed to identify measurements that discriminate among groups (Cadrin 2000). Multi-group PCA should be followed by multivariate analysis of variance (MANOVA) to confirm the presence of groups, and discriminant analysis should be followed by MANOVA, cross-validation, or, if only two groups are possible, logistic regression (Cadrin 2000; Härdle and Simar 2007).

For example, suppose that we collect 128 larvae that may represent multiple closely related taxa. Visual inspection suggests that some fish appear to have proportionally larger anal fins (group A). We measure 17 distances among 10 landmark coordinates as in the figure on the next page; distances between landmarks 4 and 6, 5 and 6, 6 and 7, 6 and 8, and 7 and 8 relate to anal fin size.

(Box continues)
**Box 9.2 Continued**

![Figure]

**Figure** Landmarks for morphometric analysis of hypothetical larval fish.

Because this analysis is exploratory (i.e., we have no prior reason based on literature, experience, or distribution to suspect that the difference in anal fin size is anything more than phenotypic variation), we perform a PCA on the 17 measured distances and retain principal components 1 and 2 for interpretation based on Horn’s test (Jackson 1993). Note the strong correlations (bolded) between distances related to the proportionally larger anal fin and the two principal components in the table below. Subsequently, MANOVA reveals statistically significant differences (Wilk’s lambda = 0.41, $F_{2,125} = 92.39$, $P < 0.001$) in measured anal fin distances between group A fish and other fish in the sample. Although not confirmatory of different species, these results suggest that the larger anal fin is outside expected variability and may warrant further investigation of these two groups of fish.

(Box continues)

Strauss and Fuiman 1985; Strauss and Bond 1990; Silva 2003). Organisms collected for these types of analyses must be fixed and preserved in such a way as to minimize, or at least standardize, shrinkage and distortion (section 9.4.1).

9.6.4.3 Other Characters

Osteological features (Dunn 1984) are often useful for identifying larval fish, and analyses based on skeletal disarticulation (Mayden and Wiley 1984), whole organism clearing and staining (Taylor 1967; Galat 1972; Brubaker and Angus 1984; Potthoff 1984; Snyder and Muth 1990; Snyder et al. 2004), and X-ray radiography (Miller and Tucker 1979; Tucker and Laroche 1984) have been employed in ichthyoplankton studies. Biochemical techniques have also been used to assist in identifying larval fishes (Leary and Booke 1990; Beckenbach 1991; Park and Moran 1994), including mtDNA (Pegg et al. 2006). Generally, these techniques have been applied to identify genetic differences between closely related species (Morgan 1975; Sidell and Otto 1978; Comparini and Rodinò 1980; Lindstrom 1999) or among stocks within a species (Heath and Walker 1987; Graves et al. 1990; Grewe et al. 1994; Sato et al. 2004).

9.6.4.4 Taxonomic Guides

Most larval fish guides and keys are limited in scope because of a lack of information on different developmental phases, regional differences in distribution, and restricted coverage of various taxonomic groups (e.g., Fuiman 1979; Fuiman et al. 1983; Nishikawa and Rimmer 1987;
Box 9.2 Continued

Table  Principal components 1 and 2 of PCA performed on 17 measured distances. Note the strong correlations (bolded) between distances related to the proportionally larger anal fin and the two principal components.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Correlation with principal component 1</th>
<th>Correlation with principal component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 2</td>
<td>0.26</td>
<td>-0.02</td>
</tr>
<tr>
<td>1 to 3</td>
<td>0.34</td>
<td>0.53</td>
</tr>
<tr>
<td>2 to 4</td>
<td>-0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>2 to 5</td>
<td>-0.19</td>
<td>0.03</td>
</tr>
<tr>
<td>3 to 4</td>
<td>0.27</td>
<td>-0.06</td>
</tr>
<tr>
<td>4 to 5</td>
<td>0.11</td>
<td>-0.04</td>
</tr>
<tr>
<td>4 to 6</td>
<td>-0.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 to 6</td>
<td>-0.28</td>
<td>0.46</td>
</tr>
<tr>
<td>5 to 8</td>
<td>-0.03</td>
<td>0.30</td>
</tr>
<tr>
<td>6 to 7</td>
<td>0.66</td>
<td>0.40</td>
</tr>
<tr>
<td>6 to 8</td>
<td>0.62</td>
<td>-0.53</td>
</tr>
<tr>
<td>7 to 8</td>
<td>-0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>7 to 9</td>
<td>-0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>8 to 9</td>
<td>0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>8 to 10</td>
<td>0.38</td>
<td>0.23</td>
</tr>
<tr>
<td>9 to 10</td>
<td>0.43</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Note the differences in scores of larvae (group A tentatively identified by anal fin length during sorting) on principal components 1 and 2 below. The distribution of these two groups warrants further investigation of possible species differences.

Figure  Distribution of scores for principal components 1 and 2 for 128 hypothetical fish.
Two of the broadest guides are the two-volume publications by Richards (2006) and Fahay (2007), which cover larval fishes of the western central and western North Atlantic oceans, respectively. Several larval fish guides include computer interactive keys (e.g., Snyder et al. 2004, 2005). Online databases are also available that cover the taxonomy, distribution, and ecology of fish early life history stages. LarvalBase (www.larvalbase.org) includes information on over 2,200 species worldwide. The Ichthyoplankton Information System is a Web-based decision support tool and database for the northeast Pacific Ocean and Bering Sea (http://access.afsc.noaa.gov/ichthyo/index.cfm). It contains information updated from Matarese et al. (1989, 2003) and includes other regional guides, taxonomic and character search capability, and illustrations to assist in larval identification. Table 9.2 provides a list of selected guides for larval fish identification.

### 9.6.5 Zooplankton Identification

Given an estimated diversity of nearly 33,000 species of marine zooplankton alone (Lenz 2000), species identification of zooplankton can be difficult. The bulk of zooplankton identification still relies on microscopes and taxonomic guides, but recent advances in imaging technology and computer software may offer significant gains in speed and precision (Benfield et al. 2007). The major groups of freshwater zooplankton are protozoans, rotifers, cladocerans, and copepods, although a few other taxa (e.g., ostracods) may sometimes be abundant in plankton samples. Limited diversities of cladocerans and rotifers are found in marine systems (e.g., Atienza et al. 2008; Wallace and Snell 2010), but juvenile and adult copepods, and adult and larval stages of a tremendous diversity of other taxa, may be abundant (e.g., Khalil and Abd El-Rahman 1997).

#### 9.6.5.1 Zooplankton Characteristics

Reproduction and early life history vary considerably among holoplanktonic organisms (those that are planktonic throughout the life cycle) and meroplanktonic organisms (those that are planktonic during only part of the life cycle). In freshwater systems, reproduction in rotifers and cladocerans is predominately parthenogenetic, whereas copepods produce fertilized eggs that develop through six naupliar and six copepodid stages (Figure 9.11A). In marine systems, many phyla (Boltovskoy 1999) have planktonic forms during ontogeny, such as trochophore larvae (polychaetes and bivalves) or nauplii, zoea, and megalopae (crustaceans). In both freshwater and marine habitats, many taxa produce dormant eggs through sexual reproduction. Dormant eggs can be extremely abundant in marine and freshwater sediments (Marcus 1990; Hairston 1996) and can remain viable for decades, yielding an important source of recolonization during periods of environmental change (Hairston 1996). Although some guides include keys that cover early development (e.g., Hudson and Lesko [2003] for Great Lakes copepod nauplii; Alekseev [2000] for copepodid instars of the Eucyclopinae in Russia; Vandekerkhove et al. [2004] for cladoceran dormant eggs in 20 European lakes), holoplankton identification is typically based on adult characteristics.

Taxonomically important structures vary considerably among planktonic organisms, ranging from easily discerned characters such as shell morphology to obscure features such as the number and position of hairs and spines on antennal or leg segments. As would be expected given the diversity of planktonic taxa in marine and freshwater systems, a tremendous variety of taxonomic characters is used to identify planktonic organisms. For example, among rotifers, the shape of the lorica (shell), ovarian number, presence and position of appendages, ciliation of the corona, and morphology of the trophi (grinding teeth) within the mastax are among the
characters most useful for identification (Figure 9.11B). For cladocerans, shell morphology and surface architecture, morphology and setal characteristics of the first and second antennae, and morphology and setation of the postabdomen and postabdominal claw are most often used to differentiate taxa (Figure 9.11C). Among copepods, body morphology, the structure of the first antennae, and setation of the caudal ramus may be diagnostic for some taxa, but identification typically relies on the morphology of the fifth leg (pereiopod), which is often difficult to discern but varies substantially among species in segment number and the position and number of spines and setae (Figure 9.11D).

9.6.5.2 Taxonomic Guides and Software

Zoogeographic information, ecological data, and morphologic characters can often be combined to identify zooplankton to species. Excellent summaries and diagrams can be found in a number of sources including Smith (2001) and Thorp and Covich (2010) for freshwater organisms and Todd et al. (1996), Boltovskoy (1999), Johnson and Allen (2005), and Young et al. (2006) for marine organisms. Generally, species-specific zooplankton identification guides are regional, whereas geographically broad texts provide coarser-level identifications (Table 9.3). Some excellent resources are found in conference proceedings, technical reports, and other “in-house” documents (e.g., Zimmerman and Hubschman 1990; Hudson and Lesko 2003). Species identi-
fication of many taxa is still problematic, as evidenced by the species-level key for cladocerans in Pennak (1989), which was followed by a genus-level key in Smith (2001), as well as both genus- and species-level identifications in Thorp and Covich (2010). For most analyses, particularly at the assemblage level, it is probably better to use a coarser taxonomic resolution (e.g., genus) with greater confidence than a finer taxonomic resolution that may sacrifice accuracy, although including tentative finer-scale identifications in a discussion of results may be important. Genetic studies have revealed considerable information on zooplankton assemblage composition, interspecific hybridization, phylogeny, and biogeographic patterns (Schwenk et al. 1998; Adamowicz et al. 2004), which will hopefully clarify taxonomic relationships and distribution patterns. Mechanical and biochemical techniques can serve as alternatives or complements to taxonomic guides (Garland and Zimmer 2002). These include scanning electron microscopy (de Schweinitz and Lutz 1976), immunofluorescence (Demers et al. 1993), flow cytometry (Legendre et al. 2001; Lorenzo-Abalde et al. 2005), and mtDNA (Bucklin et al. 2000). Automated and manual imaging systems integrated with recognition software may also help identify individual taxa (Tang et al. 1998; Benfield et al. 2007).

9.7 REFERENCES


Armstrong, J. D., and K. H. Nislow. 2006. Critical habitat during the transition from maternal provisioning in freshwater fish, with emphasis on Atlantic salmon (Salmo salar) and brown trout (Salmo trutta). Journal of Zoology 269:403–413.


Cooperman, M., and D. F. Markle. 2003. Rapid out-migration of Lost River and shortnose sucker larvae


Hoyt, R. D. 1988. A bibliography of the early life history of fishes, volumes 1 and 2. Western Kentucky University, Department of Biology, Bowling Green.


Kindschi, G. A., R. D. Hoyt, and G. J. Overmann. 1979. Some aspects of the ecology of larval fishes in
Rough River Lake, Kentucky. Pages 139–166 in R. D. Hoyt, editor. Proceedings of the third symposium on larval fish. Western Kentucky University, Department of Biology, Bowling Green.


Plankton


nature, and larval fish survival in Hauraki Gulf, New Zealand. Canadian Journal of Fisheries and Aquatic Sciences 62:593–610.


