4

Larval sampling

David K. Skelly and Jonathan L. Richardson

4.1 Introduction

Most amphibian species are metamorphic, and among those, the majority have larvae that are fully aquatic (Duellman and Trueb 1986). These larvae are found in an enormous variety of contexts ranging from bromeliads and tree holes, to brackish pools and the largest rivers and lakes (Duellman and Trueb 1986). Add to this mix of environments the fact that amphibian larvae differ dramatically in their microhabitat use, from the water surface to benthic mud, and researchers sampling larval amphibians are confronted with a non-trivial challenge of matching techniques with study goals and logistic limits. Fortunately, there is a wealth of experience that can be tapped when making decisions about where, when, and how to sample (Shaffer *et al.* 1994; Olson *et al.* 1997).

4.1.1 Why sample larvae?

The reasons to sample amphibian larvae are many, but most emphasize either strategic or logistic considerations. From the strategic perspective, larvae represent a critical life history stage. Many of the reasons that motivate scientists to study amphibians are related to the dynamics and fate of their populations. Monitoring of larval cohorts can provide critical information regarding the trajectory of a population and the factors that may affect abundance and distribution. Larvae also represent concrete evidence of breeding. While many studies of anurans use male calling as an index of breeding distribution, males can call from wetlands where no breeding takes place. Larval surveys are less likely to overestimate breeding distribution.

For many species larvae are also convenient. Many adult amphibians are found at low density spread across large areas of terrestrial habitat. In addition they are often cryptic, arboreal, or fossorial, making it extremely difficult to sample their

populations systematically. For amphibians with fully metamorphic life histories and aquatic larvae, breeding represents the point in the life cycle where a species can be indexed in a relatively small, defined area. The techniques described below offer a variety of systematic approaches to estimating population attributes that would be extremely challenging to determine for the adult stages of many amphibian species.

Because of their strategic and logistic advantages, amphibian larvae have been used in a wide range of research aimed at addressing fundamental questions in fields ranging from ecology and evolution to physiology and developmental biology. Well before amphibian larvae were widely studied by scientists concerned about declines and species extinctions, larval amphibians were held up as a model system by biologists.

4.1.2 Target responses

Once the motivation for sampling larvae is known, the target response variables may be selected (Table 4.1). Information on species occupancy is frequently sought in both basic and applied settings. Presence and absence data can be important in determining species richness within a wetland as well as providing evidence for changes in species range. Any of the techniques described in this chapter can be used to estimate species presence and absence. However, if presence/absence is the sole motivation for sampling, some techniques such as dip-netting (described below) may be much more efficient than others.

Table 4.1 Larval amphibian sampling methods and resulting response variables

Response	Metrics	Sampling methods	Inferences
Species occupancy	Presence/absence	All methods	Species distribution, richness
Relative density	Catch per unit effort (CPUE)	Time- or area-constrained sampling, trapping, litterbags	Comparing among populations and species
Density	Individuals per unit area or volume	Area- or volume-constrained sampling including box and pipe sampling	Comparing among populations and species cohort survival
Population size	Total number of individuals in population	Extrapolation from area- or volume-constrained samples, mark–recapture	Population dynamics extinction risk

Beyond estimating presence and absence, sampling can be directed at estimating density. Any technique which can be scaled by effort (sometimes expressed as catch per unit effort, or CPUE) can offer estimates of relative density; that is, the density of a given species relative to another. As one example, the number of larvae from one species recovered during 30 person minutes of dip-netting may be compared with a second species as an index of their relative density (both expressed as individuals recovered per person minute). A smaller number of techniques, identified below, allow direct estimates of the number of individuals per area, or per volume. In general, these techniques operate on the principle that a defined area or volume is sampled effectively. As one example, each use of a standard box sampler covers 0.5 m^2. After completing a set of 10 box samples within a wetland, the number of larvae of some species recovered from 5 m^2 can be expressed as the number of individuals per square meter. Finally, using mark–recapture techniques, estimates of the total number of individuals within a larval cohort may be made.

4.1.3 Timing

Amphibian larvae range from practically immobile, yolk-laden hatchlings, to 30-cm-long salamander larvae capable of moving extremely rapidly. Deciding when to sample during the larval period is best done in conjunction with decisions about how to sample. Small larvae move slowly and are often found in shallow areas. These attributes can make sampling relatively straightforward using a variety of techniques. This is something to consider if your study aims allow flexibility in the developmental stage sampled. It also suggests that effective larval sampling depends on a close knowledge of the life histories of the species to be sampled and their developmental progress within a given year. Late snowpack melt, droughts, and a particularly cold or warm spring can all shift the timing of larval development substantially. In tropical climates the time of year can be far less important than the onset of wet season rains in triggering breeding and the timing of larval sampling.

4.1.4 Sampling effort

How much is enough? In any sampling context, researchers are confronted with the task of deciding how to allocate effort. As expected, a satisfactory answer depends on study goal and tolerance of uncertainty. Any of the techniques described below can be scaled in effort. The effort adopted, however, will require direct estimates of how species detection, larval density estimates, or whatever the target variable is, changes with increased effort. In our own research, we have intentionally varied per wetland sampling effort (samples per visit, number of

visits, timing of visits) to determine the sensitivity of target responses (e.g. Werner *et al.* 2007). There is no shortcut here; rules of thumb can be misleading resulting in wasted effort and more collection than necessary or, more likely, incomplete and inaccurate information (Skelly *et al.* 2003).

If you are new to a system, plan to learn during your initial sampling. Intentionally trying multiple techniques and different degrees of sampling effort will provide information that will ultimately save you time and produce more reliable information. Before you step in the water, be prepared to spend more time in each habitat if it is needed and to consider using multiple techniques to capture the range of species and larval stages you intend to study.

4.2 Sampling techniques

4.2.1 Box/pipe sampler

4.2.1.1 Description

These area-based samplers are dropped rapidly through the water column in areas of less than 1 m in depth. The earliest versions were sheet metal or plywood boxes, 0.5 × 1.0 m, used in conjunction with a metal frame net designed to fit snugly within the width of the box (Harris *et al.* 1988). Repeated sweeps of the trapped volume of water are used to clear and count the amphibian larvae within. A second form, the pipe sampler (Skelly 1996), ranges in size but is typically constructed of an approximately 1 m length of aluminum pipe, 30 cm or so diameter. Polyvinyl-coated aluminum pipe manufactured for air handling in commercial heating and cooling applications is particularly effective. Pipe samplers are used with dip nets constructed to measure half of the diameter of the pipe. Entrapped larvae are cleared using repeated circular sweeps of the water volume (Figure 4.1a).

Researchers have developed algorithms to ensure complete, or at least consistent, clearance of animals. As one example, Werner *et al.* (2007) report sweeping the water column of a pipe sampler a minimum of 10 times, and for at least 10 null sweeps after the last collected animal has been removed from the net. For both types of sampler, multiple samples are collected in a single wetland. Researchers can use grids to lay out sampling points which can be distributed among habitat types within a wetland. Alternatively, a minimum distance between sampling points can be specified and placement of individual samples set haphazardly within that constraint. If desired, data can be kept individually for each box or pipe providing information on spatial distribution, variation in local density, patterns of species coincidence, or association with particular microenvironments (Freidenburg 2003).

Fig 4.1 Representative sampling techniques targeting larval amphibians in aquatic habitats: (a) Pipe sampling in which a pipe is used to trap larvae that are then cleared using a dip net (section 4.2.1); (b) leaf litterbags are used for sampling salamander larvae in stream habitats (from Waldron *et al.* 2003; section 4.2.4); (c) a collapsible funnel trap deployed along a pond edge (section 4.2.5); (d) a larval *Ambystoma talpoideum* salamander marked with a lateral orange visible implant elastomer (VIE) tag (photograph courtesy of Kristen Landolt of Murray State University; section 4.2.6).

4.2.1.2 Application

Box samplers are most effective within vernal ponds with an open water column and simple bottom substrates such as decaying leaves. Pipe samplers were later developed to capture the advantages of a box sampler while enabling the sampling of a wider variety of environments including those where water is interspersed with emergent vegetation (Skelly 1996). We are unaware of the use of area-based larval amphibian samplers within stream environments, although the use of comparable samplers for benthic macroinvertebrates suggests the potential for such an application.

4.2.1.3 Considerations

The major advantage of box and pipe samplers is that sampling of a known area of wetland bottom provides direct estimates of larval density (individuals/m^2). If depth within each pipe is recorded, estimates of density per unit volume can be determined. In either case, if wetland bottom area (or volume) is known, researchers have extrapolated pipe- and box-sample-based density estimates into estimates of the size of entire larval cohorts (e.g. Werner *et al.* 2007).

A major disadvantage of these area-based samplers is their time-intensiveness. Repeatedly placing and clearing a box or pipe is relatively slow and methodical work even for experienced users. In addition, when sampling takes place in remote areas that require hiking into and out of, box and pipe samplers can be inconvenient because of their size.

4.2.2 Dip net

4.2.2.1 Description

Dip nets are probably the most common sampling tools used to collect amphibian larvae. Dip-netting can be relatively unstructured if the goal is simply to capture representatives of a particular species. Alternatively, dip-net surveys in which the elapsed time (Werner *et al.* 2007) or number of sweeps (Gunzburger 2007) is counted can be used to provide estimates of relative abundance of species. With calibration from other methods such as pipe sampling, time-constrained dip-net surveys have been used to provide per-unit-area density estimates (Werner *et al.* 2007). Effective dip-netting depends strongly on the species targeted and the environment type sampled. However, most neophytes tend to collect a great deal of substrate along with each sweep. With experience, users can learn how far into the water column and substrate the net needs to pass to capture the larvae without burying them in an overabundance of mud and vegetation.

Dip nets used in larval amphibian sampling range in size and shape from small aquarium nets used in confined volumes of water (Thoms *et al.* 1997) to very large nets approaching the size of some small seines (S. Cortwright, personal communication). Regardless of net size, it is common for amphibian biologists to construct their own nets or to customize store-bought nets. Dip nets used for larval amphibian sampling can have much shallower net bags and tend to require finer mesh than those used for fish and other larger organisms. A shallow net bag facilitates rapid processing of each sweep and can speed sampling significantly. Researchers can also construct net dimensions that fit the structure of the environments they sample (e.g. narrower nets may be appropriate for dipping out of marshes with emergent vegetation, small stream pools, or tire ruts).

4.2.2.2 Application

Dip nets are used in most of the places where amphibian larvae are found. Nets can be of lighter construction in vernal ponds (e.g. using mosquito mesh for the net bag) compared with those used in streams and other places with abrasive substrates.

4.2.2.3 Considerations

Dip-netting is fast, requires a minimum of equipment, and can be performed in a wide range of environments. Nets can be constructed inexpensively to perform well in particular contexts. On the downside, as much as any technique, dip-netting effectively relies on experience. An experienced user can vastly outperform a beginner working side by side. A second disadvantage relates to population density estimates. While dip-netting can be used by itself to estimate relative density in terms of catch per unit effort (where effort is often measured as time spent sampling), estimates of area- or volume-based density must rely on other techniques or through calibration from samples collected using other methods in the same or comparable environments (Werner *et al.* 2007).

4.2.3 Seine

4.2.3.1 Description

Seines have long been used to collect amphibian larvae (Routman 1984). They are particularly effective in sampling open, deeper areas that cannot easily be sampled using box and pipe samplers or dip nets. Seining typically requires at least two people. One person at each end sets the seine in a line and then begins moving in an agreed direction until a position is reached where they move together and begin gathering the ends of the seine up and out of the water, forcing the entrapped larvae down into the middle. This is done most easily if the seine is being gathered onto the shore. Sometimes this is not possible in which case, some sort of floating platform such as a foam bucket float can be used. When the captured sample is

concentrated in the center of the seine, it is picked up and moved onto the shore where the contents are sorted and the larvae are processed as desired. Most seines used for amphibian larvae are relatively small, on the order of 3–5 m long and 1 m or so deep (Shaffer *et al.* 1994). Larger seines often have a bag sewn in the middle. The bag can greatly increase sampling effectiveness, but may also require a third person to keep it from getting caught or rolled up in vegetation.

4.2.3.2 *Application*

Seines are typically used in open-water areas of ponds and lakes, although large stream pools may also be sampled using seines in conditions where flow is not too great.

4.2.3.3 *Considerations*

Often seines are the only means of sampling larger amphibian larvae that live in open-water regions of ponds (e.g. large Ambystomatid salamander larvae). A major disadvantage of seines is that they are hard to handle in vegetation-choked ponds and lakes often frequented by larval amphibians. In such an environment, it can take over half an hour to sort through the vegetation and muck gathered in a 5-min seine haul. During this sorting process, smaller amphibian larvae can be hard to detect (and seine mesh is often coarse enough to enable their escape) meaning that seines are most often used for large larvae. As with dip-netting, seines are typically used to estimate catch per unit effort, although it is possible to estimate number captured per unit area in some conditions (Shaffer *et al.* 1994).

4.2.4 Leaf litterbags

4.2.4.1 *Description*

Leaf litterbag sampling is a relatively new method for sampling stream habitats for amphibians, particularly salamander larvae and adults (Pauley and Little 1998). Litterbags create an artificial habitat refugium reproducing leaf packs commonly found within streams, yet enclosed within mesh bags that can be easily removed and sampled for salamanders. Leaf litterbags are constructed using plastic mesh netting. Mesh gauge varies, but the 1.9 cm mesh (commonly found in deer-exclusion netting and similar products) has proven useful. The mesh is cut into squares, with 0×50, 70×70, and 90×90 cm mesh sections representing small, medium, and large bags, respectively. Several rocks are then placed on the netting to anchor the bag, followed by leaves, needles, and other material likely to be found within particular stream environment. Once filled with litter, the corners of the mesh are pulled together and cinched at the top using a cable tie (Figure 4.1b). Colored flagging may be added to facilitate retrieval; this can be critical in contexts where

the bag may be displaced downstream by a high-flow event. Litterbags tend to work best when deployed in locations where debris packs are likely to naturally accumulate (e.g. pools, channel bends). In higher-flow environments, bags can be secured by partially covering them with larger rocks or by tethering bags to roots or pinning them using a stake driven into the substrate (Waldron *et al.* 2003; Talley and Crisman 2007). Bags are sampled by placing a dip net underneath the bag in the water column, quickly removing the bag from the water, and placing it over a light-colored dishpan (Pauley and Little 1998; Jung *et al.* 2000). While gently shaking the bag, salamander larvae and adults will often fall into the dishpan where species identification and enumeration can take place. The litter pile should also be examined to ensure that all captured individuals are included. The litterbag can then be reassembled and deployed again, although the leaf litter may need to be replaced to compensate for decomposition over time.

4.2.4.2 *Application*

Litterbags have been used in stream environments where debris collects, and this technique targets species known to utilize leaf packs. They are easy to deploy, and quick and inexpensive to construct. They also manage to exploit an attribute of species that makes them otherwise hard to sample. In the absence of litterbag sampling, larvae of many stream-dwelling species are difficult to collect. They are also able to capture more secretive or uncommon species that might be missed in a dipnet survey. While litterbag sampling can produce species presence and relative density data for a stream reach, it does not likely provide accurate estimates of absolute population size or density, since it is unclear what exact area of the stream is being sampled with each bag (Chalmers and Droege 2002; Waldron *et al.* 2003).

4.2.4.3 *Considerations*

Litterbags are a highly specialized sampling tool. They are useful only for species that dwell in streams and use leaf packs. But if such a species is being targeted, the advantages of litterbags are substantial. Litterbag size is an important consideration, as medium and large bags can capture more individuals and species; however, the size of the focal stream may only accommodate a smaller bag (Waldron *et al.* 2003; Talley and Crisman 2007). Additionally, samples can be biased if potential competitors or predatory individuals colonize the bag. Researchers can discourage predatory (usually larger) adults and species by using finer mesh or submerging bags in deeper water, leading to a preferential capture of larvae (Waldron *et al.* 2003). Finally, the utility of litterbags may vary seasonally, as the abundance of natural leaf pack habitats can vary throughout the year, depending on the surrounding habitat cover.

4.2.5 Trapping

4.2.5.1 Description

Traps can be an effective means to capture amphibian larvae, requiring the researcher to simply deploy the traps and check them after a time period sufficient to have captured resident larvae. Most traps used by amphibian researchers are of a funnel design, which channels larvae into a large holding section that can be accessed by the researcher to recover captured animals. Commercially available wire minnow traps have been used in many amphibian studies (e.g. Fronzuto and Verrell 2000; Ghioca and Smith 2007). Home-made funnel traps using plastic bottles (e.g. 2-L plastic drinks bottles) have also been used successfully (Calef 1973; Richter 1995). Collapsible traps made of fine nylon mesh and available commercially (Promar, Gardena, CA, USA; Figure 4.1c), have capture rates equal to or better than traditional wire minnow traps (Adams *et al.* 1997; C. Pearl, personal communication). The finer mesh of these collapsible traps allows for the retention of much smaller larvae, and the compact size and weight make them suitable for backcountry work. Pyramid-shaped crayfish traps (Johnson and Barichivich 2004) are an alternative to minnow and collapsible mesh traps that can be particularly effective when it is important for part of the trap to extend above the water surface. In all cases, traps are deployed by dropping each in a predetermined area of the pond with a line attached and tied to a tree or float to make locating and retrieving it easier.

4.2.5.2 Application

Traps can be effective in capturing amphibian larvae present within many aquatic habitats with, perhaps, the exception of fast-moving water. Trapping is particularly suited to detection of species presence, and to estimate catch per unit effort (a metric of relative abundance). Trapping is sometimes the only suitable method in deep water, steep-sided pond basins, or frozen ponds where wading in to conduct sampling is not feasible. Additionally, it may be easier to sample habitats with structurally complex bottoms or vegetation-choked areas using traps, where seining and dip-netting may be difficult. Lastly, funnel trapping will often capture rare, secretive, or more nocturnal species not detected using other methods conducted in a short time period and during the daytime.

4.2.5.3 Considerations

Whereas traps can be left overnight, this practice requires a sampling location to be revisited within a short time period (usually 12–24 h) to avoid trap mortality, especially of non-target species or life stages. It is especially important, when traps

are to be left for extended periods, to keep part of the trap above water to allow access to water surface for trapped animals. Secondly, wire mesh size in commercial minnow traps (around 6 × 6 mm) may be too large to effectively trap smaller larvae, in which case sealing the trap with window screening composed of finer mesh or the use of nylon collapsible traps can address this issue. Also, any trapping methods and resulting data are based on an assumption of equal capture probability among individuals and populations. This can be violated if the presence of conspecifics, competitors, or predators in the cage alters capture probabilities. There could also be community-level biases if populations being sampled differ in species composition. For instance, predators present in one habitat but not the other may alter the behaviour of a target species and subsequent capture probabilities. There can be other specific sampling biases in trapping certain species and in some habitat types (e.g. playa wetlands; Ghioca and Smith 2007). Weather, larval size and developmental stage, and resource availability can all affect capture rates using traps (Adams *et al.* 1997). Some researchers use baits when trapping amphibian larvae. However, for at least some species, it appears that baiting traps does not increase trap effectiveness (Adams *et al.* 1997).

4.2.6 Mark–recapture

4.2.6.1 Description

Mark–recapture techniques are commonly used in studies of amphibian adult populations, but can also be useful for the larval stage as well. However, rapid growth often accompanied by a dramatic shift in body design can render marking methods (often developed for juvenile and adults; see Chapter 8) unsuitable for larval amphibians. Successful marking techniques for larvae include temporary injectible organic dyes (Seale and Borass 1974) and externally staining dyes, which typically stain amphibian larvae for less than 24 h (Pfennig 1999; Jung *et al.* 2002; Harris *et al.* 2003), but can also slow growth rates (Travis 1981). More permanent, yet onerous, marking techniques using paint sprays, stains, dimethyl sulfoxide, and Super Glue (Ireland 1989) have largely been replaced by more convenient and robust visible implant elastomer (VIE) tagging (Figure 4.1d). Passive integrated transponder (PIT) tags may have limited use in all but the largest larval species due to tag size (down to about 8 mm) and surgery required to implant, although the development of smaller "injectable" PIT tags, applied using a hypodermic needle, may expand the potential for this technique (Biomark, Boise, ID, USA).

VIE tagging appears to hold the most promise for amphibians, balancing the ease of marking and longevity of the actual mark. Elastomer marks consist of a silicone-based polymer material that is injected subcutaneously and cures into a

pliable and biologically inert solid (Northwest Marine Technology, Shaw Island, WA, USA). Whereas some elastomer colors are visible to the naked eye when present under translucent skin, fluorescent colors are often used and easily detected using an ultraviolet light source (portable lights are available for field purposes). Lowe (2003) used VIE to mark larvae of the spring salamander (*Gyrinophilus porphyriticus*) and has indicated that marks can still be seen 10 years after marking (W. Lowe, personal communication). Fading of the elastomer does not appear to be a common problem, although marks can migrate from the point of injection or be lost altogether. Grant (2008) found that wood frog (*Rana sylvatica*) tadpoles can retain marks through metamorphosis and that larger marks (> 2 mm) were more likely to migrate in two larval stream salamander species. Additionally, it was indicated that stream salamanders could be marked without anesthesia, while wood frog tadpoles required anesthesia and also had poorer mark retention (E. Grant, personal communication).

4.2.6.2 *Application*

Mark–recapture studies can be conducted in just about any habitat type, assuming that the same method of capture is used for each sampling period. Assuming that a sufficient proportion of the population is originally marked, mark–recapture techniques can provide robust estimates of absolute population size, especially when combined with robust capture–recapture estimation models (Chapter 24). Jung *et al.* (2002) found that mark–recapture methods provided the most accurate estimates of population size for two species of tadpoles in desert pool habitats.

4.2.6.3 *Considerations*

Regardless of technique, small amphibian larvae are relatively difficult to mark. The VIE technique will be more useful for species with larger larvae, or at least with individuals farther along in development. *Gyrinophilus porphyriticus* larvae as small as 2 cm in total length have been marked successfully, as well as *R. sylvatica* individuals down to 2.5 cm in total length. Additionally, any substantial loss of tags within a marked cohort can seriously bias population size estimates. Consider this when deciding which technique to use and for what exact purpose.

4.3 Other techniques

A number of additional techniques have been used in sampling amphibian larvae. Because they have limited application or because they have been used relatively infrequently, we mention them only briefly here and point readers to sources where more detailed descriptions are available.

4.3.1 Bottom net

A bottom net is placed on the bottom in a set position such that, when triggered, floats on an upper frame carry the net to the surface, entrapping larvae in open-water areas of small ponds (Shaffer *et al.* 1994). This area-based sampling technique has been used relatively little for amphibians, but could be effective in water where other techniques are not feasible.

4.3.2 Electroshocking

Electroshocking, developed initially to sample fish, is used primarily within streams and rivers. However, it can stun and facilitate capture of amphibian larvae as well (Brown and May 2007). It appears to be most effective for lentic species found in slow-moving parts of rivers (Shaffer *et al.* 1994). Many stream-dwelling amphibian species use retreats or bury themselves in the substrate in ways that prevent their detection and capture even if stunned during electroshocking.

4.3.3 Visual encounter survey

Visual encounter surveys, discussed more thoroughly in Chapter 15, may also be used, with some considerations, to sample larvae. Visual encounter surveys alone will likely be insufficient to detect a significant proportion of larvae present in dark or turbid water (such as a tannin-rich vernal pool, an algae-/vegetation-choked pond, or a stream with turbulent waters). Larval behavior and microhabitat preference (e.g. aggregation, crypsis, water-column basking, hiding in the substrate, preference for deep water or thick vegetation) can lead to species-specific and highly variable rates of detection. However, where water conditions allow, and the potential for confusing species is low, visual encounter surveys can be an efficient technique.

4.4 Conclusions

As in many aspects of field biology, the techniques used for sampling amphibian larvae are often passed without criticism or comment from one generation of researchers to the next. In many cases, there has been little effort to ask why one technique should be used as opposed to its alternatives or how a given technique may be most effectively applied. The many techniques outlined in this chapter are connected through the references listed below to an enormous cumulative effort to understand the most effective and efficient means to estimate the presence and density of larvae. Most of the techniques require little equipment, and that equipment is typically relatively inexpensive. Neither are the techniques difficult to master. Collectively, this means that there is little

reason not to try multiple techniques and to calibrate and understand the consequences of altering the timing and intensity of sampling. The modest effort to do so will greatly increase the reliability of the information gathered and, in all probability, lead to unforeseen insights into the biology of the species being studied.

4.5 Acknowledgments

We thank S. Cortwright, M. McPeek, E. Werner, and H. Wilbur for teaching us about larval amphibian sampling. M. Adams, E. Grant, B. Hossack, W. Lowe, and C. Pearl provided helpful insight and details into the techniques they use.

4.6 References

Adams, M.J., Richter, K.O., and Leonard, W.P. (1997). Surveying and monitoring amphibians using aquatic funnel traps. In D.H. Olson, W.P. Leonard, and R. Bury (eds), *Sampling Amphibians in Lentic Habitats: Methods and Approaches for the Pacific Northwest: Northwest Fauna Number 4*, pp. 47–54. Society for Northwestern Vertebrate Biology, Olympia, WA.

Brown, L.R. and May, J.T. (2007). Aquatic vertebrate assemblages of the Upper Clear Creek Watershed, California. *Western North American Naturalist*, **67**, 439–51.

Calef, G.W. (1973). Natural mortality of tadpoles in a population of *Rana aurora*. *Ecology*, **54**, 741–58.

Chalmers, R.J. and Droege. S. (2002). Leaf litter bags as an index to populations of northern two-lined salamanders (*Eurycea bislineata*). *Wildlife Society Bulletin*, **30**, 71–4.

Duellman, W.E. and Trueb, L. (1986). *Biology of Amphibians*. John Hopkins University Press, Baltimore, MD.

Freidenburg, L.K. (2003). *Spatial Ecology of the Wood Frog (Rana sylvatica)*. PhD Dissertation, University of Connecticut, Storrs, CT.

Fronzuto, J. and Verrell, P. (2000) Sampling aquatic salamanders: tests of the efficiency of two funnel traps. *Journal of Herpetology*, **34**, 146–7.

Ghioca, D.M. and Smith, L.M. (2007). Biases in trapping larval amphibians in playa wetlands. *Journal of Wildlife Management*, **71**, 991–5.

Grant, E.H.C. (2008). Visual implant elastomer mark retention through metamorphosis in amphibian larvae. *Journal of Wildlife Management*, **72**, 1247–52.

Gunzburger, M.S. (2007). Evaluation of seven aquatic sampling methods for amphibians and other aquatic fauna. *Applied Herpetology*, **4**, 47–63.

Harris, R.N, Alford, R.A., and Wilbur, H.M. (1988). Density and phenology of *Notophthalmus viridescens dorsalis* in a natural pond. *Herpetologica*, **44**, 234–42.

Harris, R.N., Vess, T.J., Hammond, J.I., and Lindermuth, C.J. (2003). Context-dependent kin discrimination in larval four-toed salamanders *Hemidactylium scutatum* (Caudata: Plethodontidae). *Herpetologica*, **59**, 164–77.

Ireland, P.H. (1989). Larval survivorship in two populations of *Ambystoma maculatur Journal of Herpetology*, **23**, 209–15.

Johnson, S.A. and Barichivich, W.J. (2004). A simple technique for trapping *Siren la ertina*, *Amphiuma means*, and other aquatic vertebrates. *Journal of Freshwater Ecolog* **19**, 263–9.

Jung, R.E., Droege, S., Sauer, J.R., and Landy R.B. (2000). Evaluation of terrestri and streamside salamander monitoring techniques at Shenandoah National Par *Environmental Monitoring and Assessment*, **63**, 65–79.

Jung, R.E., Dayton, G.H., Williamson, S.J., Sauer, J.R., and Droege, S. (2002). A evaluation of population index and estimation techniques for tadpoles in desert pool *Journal of Herpetology*, **36**, 465–72.

Lowe, W.H. (2003). Linking dispersal to local population dynamics: a case study using headwater salamander system. *Ecology*, **84**, 2145–54.

Olson, D.H., Leonard, W.P., and Bury, R.B. (eds) (1997). *Sampling Amphibians in Lent Habitats: Methods and Approaches for the Pacific Northwest: Northwest Fauna Number* Society for Northwestern Vertebrate Biology, Olympia, WA.

Pauley, T.K. and Little, M. (1998). A new technique to monitor larval and juvenile sal manders in stream habitats. *Banisteria*,**12**, 32–6.

Pfennig, D.W. (1999). Cannibalistic tadpoles that pose the greatest threat to kin are mo likely to discriminate kin. *Proceedings of the Royal Society of London Series B Biologic Sciences* **266**, 57–61.

Richter, K.O. (1995). A simple aquatic funnel trap and its application to wetland amphil ian monitoring. *Herpetological Review*, **26**, 90–1.

Routman, E.J. (1984). A modified seining technique for single person sampling of deep cold water. *Herpetological Review*, **15**, 72–3.

Seale, D. and Borass, M. (1974). A permanent mark for amphibian larvae. *Herpetologic* **30**, 160–2.

Shaffer, H.B., Alford, R.A., Woodward, B.D., Richards, S.J., Altig, R.G., and Gascon, C (1994). Quantitative sampling of amphibian larvae. In W.R. Heyer, M.A. Donnell R.W. McDiarmid, L.C. Hayek, and M.S. Foster (eds), *Measuring and Monitorir Biological Diversity: Standard Methods for Amphibians*, pp. 130–41. Smithsonia Institution Press, Washington DC.

Skelly, D.K. (1996). Pond drying, predators, and the distribution of *Pseudacris* tadpole *Copeia*, **1996**, 599–605.

Skelly, D.K., Yurewicz, K.L., Werner, E.E., and Relyea, R.A. (2003). Estimating declir and distributional change in amphibians. *Conservation Biology*, **17**, 744–51.

Talley, B.L. and Crisman, T.L. (2007). Leaf litterbag sampling for larval plethodont salamander populations in Georgia. *Environmental Monitoring and Assessment*, **13** 509–15.

Thoms, C., Corkran, C.C., and Olson, D.H. (1997). Basic amphibian survey for inver tory and monitoring in lentic habitats. In D.H. Olson, W.P. Leonard, and R. Bur (eds), *Sampling Amphibians in Lentic Habitats: Methods and Approaches for the Pacif Northwest: Northwest Fauna Number 4*, pp. 35–46. Society for Northwestern Vertebra Biology, Olympia, WA.

Travis, J. (1981). The effect of staining on the growth of *Hyla gratiosa* tadpoles. *Copeia*, **1981**, 193–6.

Waldron, J.L., Dodd, Jr., C.K., and Corser, J.D. (2003). Leaf litterbags: factors affecting capture of stream-dwelling salamanders. *Applied Herpetology*, **1**, 23–36.

Werner, E.E., Skelly, D.K., Relyea, R.A., and Yurewicz, K.L. (2007). Amphibian species richness across environmental gradients. *Oikos*, **116**, 1697–1721.